BIOLOGICAL HYDROGEN PRODUCTION BY USING CO-CULTURES OF PNS BACTERIA

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BIOLOGICAL HYDROGEN PRODUCTION BY USING CO-CULTURES OF PNS BACTERIA

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ABSTRACT

BIOLOGICAL HYDROGEN PRODUCTION BY USING CO-CULTURES OF PNS BACTERIA

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Biological hydrogen production is a renewable, carbon-neutral and clean route for hydrogen production. Purple non-sulfur (PNS) bacteria have the ability to produce biohydrogen via photofermentation process. The type of the bacterial strain used in photofermentation is known to have an important effect on hydrogen yield. In this study, the effect of different co-cultures of PNS bacteria on photofermentation process was investigated in search of improving the hydrogen yield.

For this purpose, growth, hydrogen production and substrate utilization of single and co-cultures of different PNS bacteria (*R. capsulatus* (DSM 1710), *R. capsulatus* hup-

(YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864)) were compared on artificial H_2 production medium in 150 mL photobioreactors under continuous illumination and anaerobic conditions.

In general, higher hydrogen yields were obtained via co-cultivation of two different PNS bacteria when compared with single cultures. Further increase in hydrogen yield was observed with co-cultivation of three different PNS bacteria.

Co-cultures of two different PNS bacteria have resulted in up to 1.4 and 2.1 fold increase in hydrogen yield and hydrogen productivity.

Whereas co-cultures of three different PNS bacteria have resulted in up to 1.6 and 2.0 fold increase in hydrogen yield and hydrogen productivity compared to single cultures.

These results indicate that, defined co-cultures of PNS bacteria produce hydrogen at a higher yield and productivity, due most probably to some synergistic relationship. Further studies regarding the physiological and molecular changes need to be carried out for deeper understanding of the mechanism of hydrogen production in co-cultures.

Keywords: Purple Non-Sulfur Bacteria, Co-cultivation, Biological Hydrogen Production

ÖZ

PNS BAKTERİLERİNİN EŞ-KÜLTÜRLERİ KULLANILARAK BİYOLOJİK HİDROJEN ÜRETİMİ

Baysal, Görkem
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Biyolojik hidrojen üretimi, yenilenebilir, karbon nötral ve temiz bir hidrojen üretim yoludur. Mor Kükürtsüz (PNS) bakteriler fotofermentasyon işlemi ile hidrojen üretebilme yeteneğine sahiptirler. Fotofermentastyonda kullanılan bakterilerin hidrojen verimliliği üzerinde önemli bir etkisi olduğu bilinmektedir. Bu çalışmada, mor, kükürtsüz bakterilerin farklı eş kültürlerinin hidrojen verimliliğini artırmaya yönelik olarak fotofermentasyon sürecine etkileri çalışılmıştır.

Bu amaç ile; farklı mor, kükürtsüz bakterilerin (*R. capsulatus* (DSM 1710), *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127) ve *R. sphaeroides* O.U.001 (DSM 5864)) tek ve eş kültürlerinin büyüme, hidrojen üretimi ve substrat kullanımları 150

mL'lik fotobiyoreaktörlerde, yapay hidrojen üretim besi yerinde, sürekli aydınlatma altında ve anaerobik koşullarda karşılaştırılmıştır.

Genel olarak iki farklı suş mor, kükürtsüz bakterinin eş kültürü vasıtasıyla tek kültürlerine göre daha yüksek hidrojen verimliliğine ulaşılmıştır. Üç farklı mor kükürtsüz bakterinin eş kültür yöntemi ile kullanılması ise, hidrojen verimliliğini daha da artırmıştır.

Farklı iki PNS bakterisinin eş kültivasyonu, 1,4 kata kadar hidrojen verimliliği artışı ve 2,1 kata kadar hidrojen üretkenliği artışı sağlamıştır.

Farklı üç PNS bakterisinin eş kültivasyonu ise, tek kültüre göre 1,6 kata kadar hidrojen verimliliği artışı ve 2,0 kata kadar hidrojen üretkenliği artışı sağlamıştır.

Bu sonuçlar, mor, kükürtsüz bakterilerin tanımlı eş kültürlerinin büyük olasılıkla bazı sinerjistik ilişkiler sebebiyle daha yüksek verimlilik ve üretkenlik ile hidrojen üretebileceğini öne sürmüştür. Eş kültürlerin hidrojen üretim mekanizmalarını daha derin bir şekilde anlamak için, fizyolojik ve moleküler değişimlere dair daha fazla çalışma yapılmalıdır.

Anahtar Kelimeler: Mor Kükürtsüz Bakteriler, Eş Kültür, Biyolojik Hidrojen Üretimi

This study has been dedicated to the hope of a future,

In which the economy is not based on the selection of scarce resources

Instead of the renewable ones

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LIST OF SYMBOLS AND ABBREVIATIONS

ADP	Adenosine di-Phosphate
ATP	Adenosine tri-Phosphate
COD	Chemical Oxygen Demand
C. saccharolyticus	Caldicellulosiruptor saccharolyticus
Fd	Ferredoxin
GC	Gas Chromatography
gdcw	Gram dry cell weight of bacteria
HPLC	High Pressure Liquid Chromatography
hup-	Uptake Hydrogenase Deficient
Lc	liter-culture
NAD	Nicotineamid Adenine Dinucleotide
PBR	Photobioreactor
PHB	Poly-β-hydroxy butyrate
Pi	Inorganic Phosphate
PNS	Purple Non-Sulfur
PFL	Pyruvate formate lyase
PFOR	Pyruvate ferredoxin (flavodoxin) oxidoreductase

CHAPTER 1

1. INTRODUCTION

Fossil fuels supplies about 80 % of the energy requirements today. However fossil fuel sources are expected to be depleted in the near future. Additionally, burning of fossil fuels cause carbon dioxide emission to the atmosphere, which acts as a greenhouse gas, burning of fossil fuels contributes to global warming.

Due to these facts; new, clean and renewable energy sources are required. Hydrogen is accepted as a clean fuel due to formation of water as end product during combustion, hence is considered as a very important potential fuel type for the future.

Biological hydrogen production is the promising route among hydrogen production methods, since it is the cleanest and the renewable hydrogen production method. Utilization of sunlight and waste waters is able to render the process more environment friendly and economical.

Biological hydrogen production can be actualized in two steps as fermentative and photosynthetic processes, which could be integrated for higher efficiency. Fermentative process requires wastes containing glucose or sucrose, whereas photosynthetic process utilizes much smaller molecules such as organic acids, which are also the by-products of the fermentative process. Purple non-sulfur photosynthetic bacteria are used for photosynthetic hydrogen production. *R. capsulatus* (DSM 1710), *R. capsulatus* hup-

(YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) are such purple, non-sulfur, photosynthetic bacteria which have been previously experimented for biological hydrogen production in METU Hydrogen Research Laboratory.

This study is aimed to compare the efficiencies of co-cultures of two and three different strains of PNS bacteria: *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) with single cultures of the same bacteria in the photosynthetic steps of the biological hydrogen production.

1.1 Hydrogen

Hydrogen is the chemical element, having atomic number 1 and an average atomic weight of 1.008 amu. It is the lightest and the most abundant element, constituting about 75 % of the universe (Das, Veziroğlu, 2001), which is denoted by symbol "H". Hydrogen gas is the two atom molecule of hydrogen atoms, with chemical formula of H_2. Hydrogen gas is colorless, odorless, tasteless, non-toxic non-metallic and is highly combustible. Although hydrogen is the most abundant element itself, the diatomic H_2 gas is rarely found, because hydrogen atoms usually form hydrides such as borohydrides (BH_3, B_2H_6), alumino hydrides (AlH_3), magnesium hydrides (MgH_2) and sodium hydrides (NaH) and covalent compounds with other elements such as water and organic compounds. The boiling point of H_2 is -253 °C, and its density is 0.09 g/L. Combustion of 1 kg of hydrogen gas leads to 142 kJ, the same energy resulting from combustion of 2.1 kg of natural gas, or 2.8 kg of gasoline (BACAS Report 2006, www.eren.doe.gov/hydrogen), which shows that hydrogen gas has very high energy to mass ratio, thus its importance as an energy carrier is significant.

The combustion reaction of hydrogen gas is shown in Equation (1.1).

$$2\ H_2\ (g) + O_2 \rightarrow 2\ H_2O\ (l) + 286\ kJ/mol \qquad (1.1)$$

As shown in the equation, combustion of hydrogen gas leads to water formation as product hence is considered as a clean energy carrier.

Hydrogen is utilized today in petroleum industry for hydrocracking and hydrodesulfurization, in chemical industry for ammonia and methanol production, in food industry for hydrogenated oil production, in welding as a shielding gas, in electricity production as a coolant gas, in nuclear fission as reactant, and in rocket engines and fuel cells as fuel (Veziroğlu, 1995, Czuppon et al.,1996, Ramachandran et al., 1998). Moreover it is the best fuel for fuel cells, a type of battery, which generate electricity directly (Şengül et al., 2009, Bayrakçelen et al., 2008).

1.2 Hydrogen Production Methods

As a promising energy carrier, and a current requirement of today's industry, hydrogen can be produced with a variety of routes such as: hydrogen waste stream, steam reforming, partial oxidation, plasma reforming, coal gasification, electrolysis and thermolysis, sulfur-iodine cycle, extraction from urine, fermentative hydrogen production, enzymatic hydrogen production and biocatalysed electrolysis. These chemical, electrolytic, photolytic, thermo-chemical, and biological methods utilize various sources including fossil resources such as natural gas, petroleum, coal; renewable resources such as biomasses; and water (Riis et al., 2005, Holladay et al.,

2009). The main hydrogen production methods were summarized below:

Steam reformation from natural gas is the technique, through which most of the hydrogen is produced today. For this technique, natural gas and steam is passed through nickel based catalysts, at temperatures between 650-700 °C, and hydrogen and carbon monoxide mixtures are produced. Lighter hydrocarbons such as naphtha can also be used for hydrogen production with steam reformation. It is the most economical way of hydrogen production, however for higher efficiencies (exceeding 80 %), larger reformers (with a capacity to produce 100,000 tones of hydrogen per year) are required (BACAS Report, 2006, Mc. Auliffe, 1980).

Steam reformation reactions are given in Equation (1.2):

$$CH_4 + H_2O \rightarrow CO + 3H_2 \text{ (steam reforming)} \qquad (1.2)$$
$$CO + H_2O \rightarrow CO_2 + H_2 \text{ (water-gas shift reaction)}$$

Partial oxidation of hydrocarbons is performed by partial combustion of fuel-air mixture in a reformer. Syngas containing hydrogen and carbon monoxide is evolved as the product. The Equation (1.3) for partial oxidation reaction is given below.

$$C_nH_m + n/2\ O_2 \rightarrow n\ CO + m/2\ H_2 \qquad (1.3)$$

Coal gasification is another widely used technique for hydrogen production. For this technique, coal is reacted with oxygen and steam under high pressure and temperatures exceeding 700 °C. Hydrogen and carbon monoxide, mixed with carbon dioxide and

steam are formed as product. Since coal is abundantly found on the globe, this technique is also convenient; however due to carbon dioxide and carbon monoxide evolution, it could not be regarded as a clean method. The Equations (1.4) for coal gasification reaction is given below.

$$3C + O_2 + H_2O \rightarrow H_2 + 3\,CO \text{ (coal gasification)} \qquad (1.4)$$
$$CO + H_2O \rightarrow CO_2 + H_2 \text{ (water-gas shift reaction)}$$

Another hydrogen production method, which is industrially applied currently, is electrolytic splitting of water into H_2 and O_2. This technique provides hydrogen gas with high purity. It is mainly applied in small scale production facilities. One advantage of utilizing this technique is the ability to use clean and renewable sources for the production of the required electricity such as transformation from solar, wind, hydro power. However the required electricity for this technique is relatively high. The reaction for electric splitting of water is given below in Equation (1.5)

$$2\,H_2O(l) \rightarrow 2\,H_2(g) + O_2(g) \qquad (1.5)$$

Among various sources and routes, today 48 % of the hydrogen production is from natural gas, 30 % is from petroleum, which is mostly used in petroleum refineries itself, 18 % is from coal, 4 % is produced via electrolysis, and only 1 % is produced from biomass, which includes pyrolysis, gasification and biological hydrogen production methods (www.eren.doe.gov/hydrogen) (Figure 1.1). Among hydrogen production methods from biomass, the biological method is the less energy intensive, environmentally benign and renewable one since agroindustral wastes, waste waters and

sunlight can be utilized. Hence emphasis on biological hydrogen production is crucial for a cleaner future, not to be used as an energy carrier only, but also for cleaner actualization of supplying the hydrogen demand of even today.

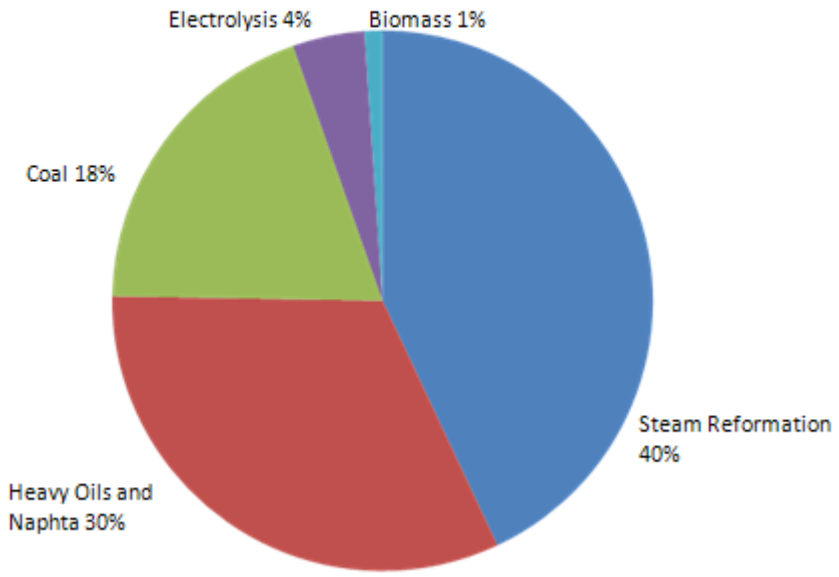


Figure 1.1: Hydrogen production ratios by methods

1.3 Biological Hydrogen Production

The hydrogen production methods carried out via algae, cyanobacteria, fermentative bacteria, and photosynthetic bacteria are classified as biological hydrogen production (Nandi and Sengupta, 1998).

Biological hydrogen production can also be further divided into following classes (Das

and Veziroğlu, 2001):

-Biophotolysis of Water

-Fermentative Hydrogen Production Method

-Photofermentation Method

-Integrated Systems

1.3.1 Biophotolysis of Water

Biophotolysis of water is the process of splitting water molecules to hydrogen and oxygen by algae and cyanobacteria under anaerobic conditions via utilization of light as driving force (Gaffron and Rubin, 1992). The total biophotolysis reaction by algea, which is catalyzed by [FeFe]-hydrogenase is shown in Equation (1.6).

Direct biophotolysis:

$$2\ H_2O\ (l) + \text{light} \rightarrow 2\ H_2\ (g) + O_2\ (g) \tag{1.6}$$

The total biophotolysis reaction by cyanobacteria, which is catalyzed by [NiFe]-hydrogenase is shown in Equation (1.7).

Indirect biophotolysis:

$$12H_2O(g) + CO_2 \rightarrow C_6H_{12}O_6(g) + 6O_2(g) \tag{1.7}$$

$$C_6H_{12}O_6(g) + H_2O(g) \rightarrow 12H_2(g) + 6CO_2\ (g)$$

The efficiency of hydrogen production via biophotolysis is low, besides since the biophotolysis reaction requires anaerobic environment, produced oxygen reduces the

reaction rate by inhibition of nitrogenase and hydrogenase enzymes (Yu and Takashi, 2007).

1.3.2 Dark Fermentation

Fermentative hydrogen production, which is also called dark fermentation, is the process of conversion of organic compounds such as glucose and sucrose to hydrogen, carbon dioxide and lower weight organic compounds such as organic acids like acetate and butyrate by anaerobic bacteria, in the absence of light. (Figure 1.2) The dark fermentation reaction which is catalyzed by hydrogenases is shown in Equation (1.8).

$$C_6H_{12}O_6\ (l) \rightarrow H_2\ (l) + CO_2\ (l) + \text{Organic acids} \qquad (1.8)$$

Clostridium butyricum, Clostridium acetobutyricum, Clostridium beijerinckii, Clostridium thermolacticum, Clostridium saccharoperbutylacetonicum, Clostridium tyrobutyricum, Clostridium thermocellum and *Clostridium paraputrificum*) (Chong et al., 2009) and *Caldicellulosiruptor saccharolyticus* (Vrije et al., 2007) are among strains of bacteria which are utilized for hydrogen production by dark fermentation.

If we consider the advantages and disadvantages of dark fermentation, one of the advantages of dark fermentation is that, direct solar input is not required and hence reactors are simple (Hallenbeck and Ghosh, 2009). Also, different types of wastewaters and agricultural wastes can be utilized for dark fermentation reaction (Kapdan and Kargi, 2006). Besides, valuable side products are formed during dark fermentation reaction such as acetic acid, butyric acid and lactic acid. However, due to incomplete

degradation of organic compounds, hydrogen yields of the dark fermentation are low (Hallenbeck and Ghosh, 2009).

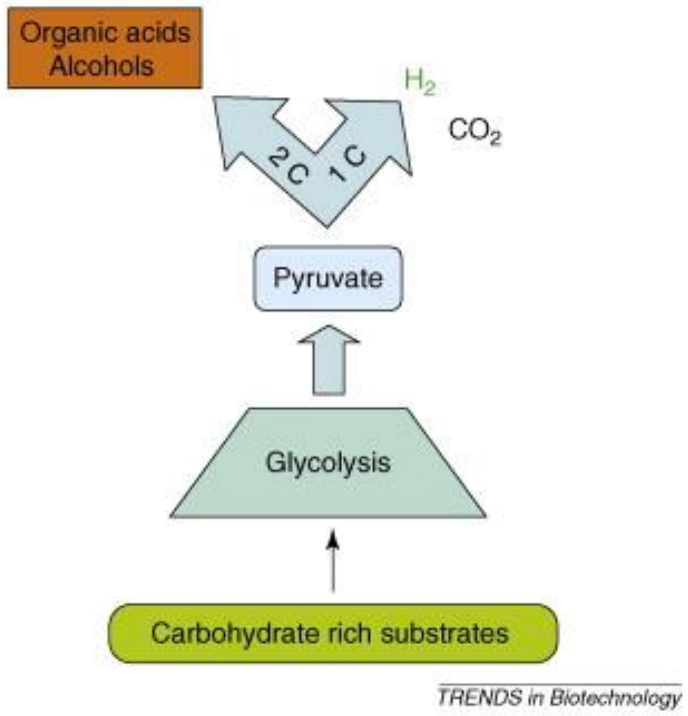


Figure 1.2: General scheme of dark fermentation (Hallenbeck and Ghosh, 2009)

1.3.3 Photofermentation

Photofermentative hydrogen production is a method in which photosynthetic bacteria, which can break down organic acids into hydrogen and carbon dioxide in the presence of light, are utilized. (Figure 1.3) The overall photofermentative H_2 production from acetate reaction is shown in Equation (1.10).

$$CH_3COOH + 2\ H_2O\ (l) + light \rightarrow 4\ H_2\ (g) + 2\ CO_2\ (g) \qquad (1.10)$$

Hydrogen is produced by photosynthetic bacteria via light dependent electron transfer in nitrogen limited and anaerobic environments (Özgür, Mars et al., 2010).

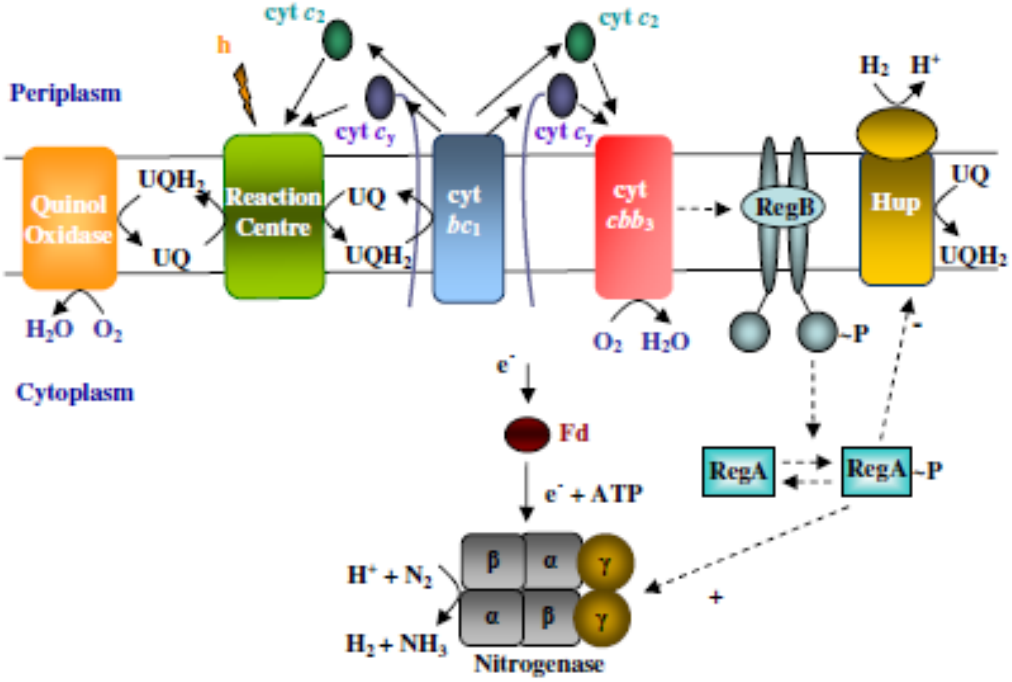


Figure 1.3: General scheme of photofermentation (Öztürk et al., 2005)

In order to produce biohydrogen by photofermentation method, purple non-sulfur bacteria (such as *Rhodobacter* or *Rhodopseudomonas* species including *R. capsulatus*, *R. palustris* and *R. sphaeroides*) or purple sulfur bacteria (such as *Chromatium* or *Thiocapsa* species) are utilized (Basak and Das, 2007, Kovacs et al., 2000).

The advantages of photofermentation method when compared with direct photolysis are listed below (Das and Veziroğlu, 2001):

i) Theoretical conversion yields are high,
ii) Since anaerobic photosynthesis does not lead to oxygen formation, the process in not inhibited by the presence of oxygen,
iii) Broad spectrum of light can be utilized for application,
iv) Wide variety of organic substrates including organic acids can be utilized from different wastes including food processing wastes and wastewaters and agricultural wastes.

The main drawbacks of photofermentative hydrogen production are the low H_2 productivity and high photobioreactor costs. Additionally development of photobioreactors which are permissive to light but not permissive to oxygen is mandatory (Fedorov et al., 1998). Also light conversion efficiencies are low and energy demand by nitrogenase is high (Hallenbeck and Ghosh, 2009). Further investigation has to be performed in order to increase the efficiency and feasibility of the process. Detailed information regarding photofermentative H_2 production method is given in section 1.4.

1.3.4 Integrated Systems

The fermentative hydrogen production method utilizes organic substrates such as glucose and sucrose, and produces hydrogen and organic acids, and photosynthetic hydrogen production method utilizes organic acids as substrates. Hence fermentative hydrogen production method and photosynthetic hydrogen production method can be

combined in order to maximize the production of hydrogen from the same amount of organic substrate utilized for fermentative method only. Therefore the organic acids, which are the by-products of the fermentative hydrogen production method, can be further utilized for more hydrogen production, increasing the yield.

This approach has been utilized by an EU 6th framework integrated project: HYVOLUTION – Non-thermal production of pure hydrogen from biomass (Classen and Vrije, 2006) Figure 1.4 summarizes the general scheme of the HYVOLUTION project.

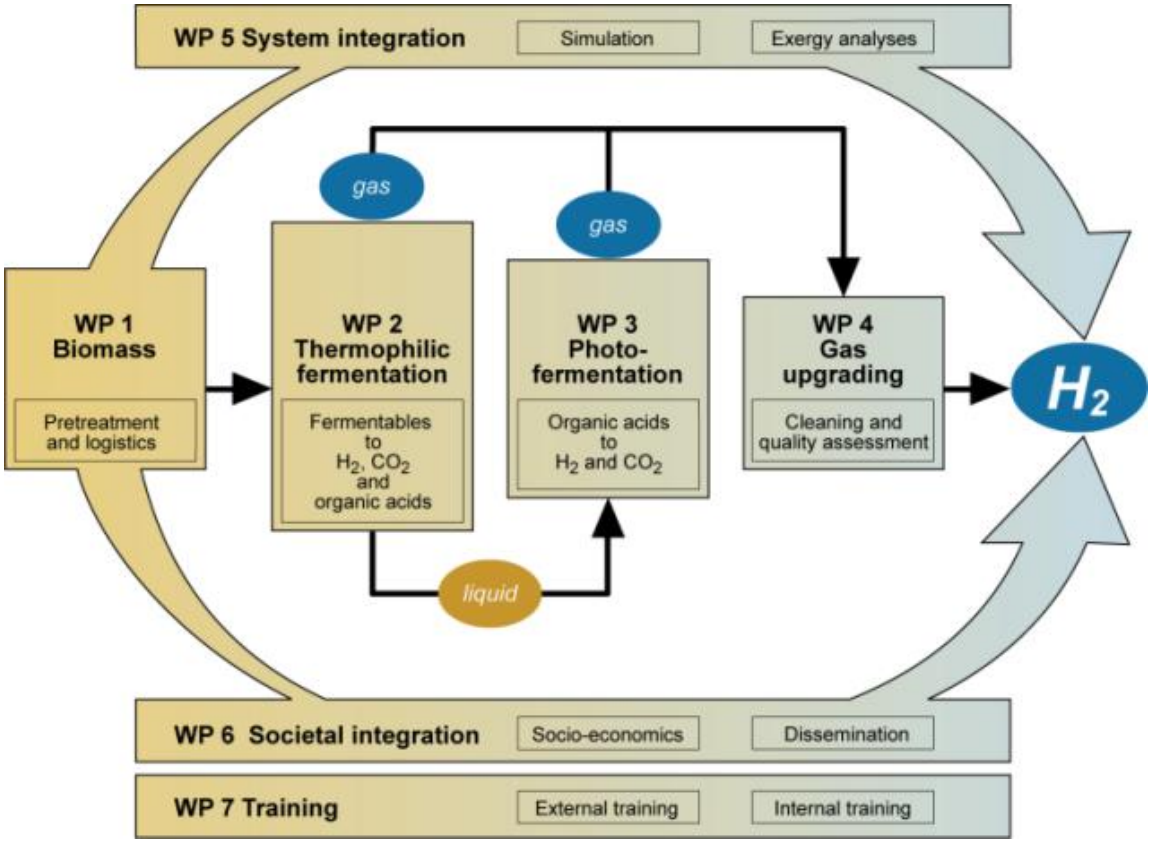


Figure 1.4: General Scheme of HYVOLUTION Project

HYVOLUTION project has focused on the development of an integrated cost effective pure hydrogen production method from multiple biomass feedstocks, targeting 75 % efficiency via production of nine moles of hydrogen per mole of hexose consumed (Classen and Vrije, 2006). Within the scope of HYVOLUTION, the first step of hydrogen production was thermophilic fermentation (dark fermentation) of various feedstocks including molasses, potato steam peel and thick juice. In the second step, the effluents of thermophilic fermentation containing organic acids (mainly acetate) were converted to hydrogen by photofermentation (http://www.biohydrogen.nl). METU Biohydrogen Group was a member of HYVOLUTION project and has coordinated the work package 3 (WP3), photofermentation.

1.4 Photofermentative Hydrogen Production

1.4.1 Purple Non-Sulfur Bacteria

Purple non-sulfur bacteria are ovoid to rod shaped, aquatic, photosynthetic, gram negative prokaryotes, which grow at a pH range between 6 and 9, and a temperature range between 25 and 35 °C. They divide by binary fission. Although PNS bacteria have vesicular photosynthetic membranes, they differ from plants and algae, by not having photosystem II. They are found in various habitats such as freshwater, marines, and soil. (Sasikala et al., 1993, Imhoff et al., 1984). PNS bacteria, which are also microaerophilic, are able to survive in dark and light conditions (Biebl and Pfenning, 1981).

Their color is brown, varying between light greenish brown to dark brown, although upon exposure to air, their color can turn into red (Holt et al., 1984). Conversion of carotenoids to ketocarotenoids is responsible for the color change (Pellerin and Gest,

1983). Purple non-sulfur bacteria can favor aerobic chemoautotrophic, aerobic chemoheterotrophic, anaerobic phototrophic and anaerobic photoheterotrophic growth modes depending on the physiological conditions (Table 1.1). Autotrophic growth is favored when carbon dioxide is the carbon source, whereas heterotrophic growth is favored when organic acids are the carbon source. Light is required for phototrophic and photoheterotrophic growth modes (Basak and Das, 2007, Koku, 2002). Purple non-sulfur bacteria, which lack photosystem II, can not split water, hence able to perform anoxygenic photosynthesis, which makes them valuable organisms for biological hydrogen production. Photoheterotrophic growth is the suitable growth mode for biological hydrogen production applications, and addition of one or more water soluble vitamins are necessary for phototrophic growth mode (Sasikala et al., 1991).

PNS bacteria can not utilize hydrogen sulfide as an electron donor; hence they are called "Non-sulfur" bacteria. However PNS bacteria can utilize sulfide, although still in lower concentrations than it is utilized by sulfur bacteria (Pellerin and Gest, 1983).

PNS bacteria also produce valuable by-products including Poly-β-hydroxy butyric acid (PHB) and carotenoids (Uyar, 2008). PHB is a biodegradable thermoplastic which is produced by the photosynthetic bacteria especially when carbon sources are present in excess amounts; and nitrogen, sulfur, or phosphorus sources are limited. PHB is produced as a storage material for the cell, by photosynthetic bacteria (Hustede et al., 1993). Apart from PHB and carotenoids, the cell biomass itself is a valuable end-product since it can be utilized as a fertilizer.

Table 1.1: Modes of Growth for *R. sphaeroides* (Koku et al., 2002)

Mode of growth	Carbon source	Energy source	Notes and relation to hydrogen production	Example strains
Photoheterotrophy	Organic carbon	Light	Preferred growth mode by PNS bacteria. Only mode resulting in hydrogen production.	O.U. 001
Photoautotrophy	Inorganic carbon (CO_2)	Light	In the absence of organic carbon. Results in consumption of hydrogen.	2.4.1
Aerobic respiration (chemoheterotrophy)	Organic carbon	Organic carbon	In the presence of oxygen. Stops hydrogen production.	O.U. 001
Anerobic respiration (chemoheterotrophy)	Organic carbon	Organic carbon	Under anaerobic, low light availability conditions. No hydrogen production. Marginal growth.	sp. denitrificans
Fermentation	Organic carbon	Organic carbon	Under anaerobic-dark conditions. No hydrogen production. Allows marginal growth.	ATCC 17023; R26; 2.4.16

Different strains of PNS bacteria were utilized for photofermentative hydrogen production including *R. capsulatus*, *R. palustris* and *R. sphaeroides* (Basak and Das, 2007, Hustede et al., 1993, Kondo et al.,2002, Okubo, 2007, Saskala, 1991, Sasikala 1994, Özgür, Uyar et al., 2010, Eroğlu et al., 2010, Özgür, Mars et al., 2010, Akköse et al., 2009, Uyar et al., 2008, Eroğlu et al., 2009, Kars et al., 2009, Kars et al., 2008, Eroğlu et al. 2008). Co-cultures of *R. capsulatus*, *R. palustris* and *R. sphaeroides* for hydrogen production from sugar beet molasses was also investigated by another study

(Sağır, 2012). However a study investigating the performances of co-cultures PNS bacteria on artificial media has been lacked. Here, the most widely used PNS strains will be introduced, briefly.

***Rhodobacter capsulatus*:**

Rhodobacter capsulatus is a rod-shaped, photosynthetic gram-negative purple non-sulfur bacteria. Its cell diameter ranges between 0.5 and 1.2 µm. It multiplies by binary fission via producing capsules and slime. The cells have polar flagella, which functions in motility (Imhoff et al., 1995). *R. capsulatus* requires thiamine, and in certain media biotin and nicotinic acid as growth factors (Koser, 1948). It has vesicular photosynthetic membranes and grows optimally between pH 6 and 9, and temperature between 25°C and 35°C (Sasikala et al., 1993). It can live in the presence and absence of light, in aerobic and anaerobic conditions (Biebl and Pfennig, 1981). *R. capsulatus* can enter one of the five growth modes; photoautotrophic, photoheterotrophic, aerobic chemoorganotrophic, fermentative chemoorganotrophic and chemolithotrophic (Klipp et al., 2004). It can exhibit yellowish brown to greenish deep brown color, however in the presence of oxygen, turns into red due to conversion of carotenoids to ketocarotenoids (Pellerin and Gest, 1983). *R. capsulatus*' bacteriochlorophyll *a* has absorption values of 376-378, 450-455, 478-480, 508-513,590-592, 802-805 and 860-863 nm (James et al., 2005). Microscopic images of *R. capsulatus* are given in Figure 1.5.

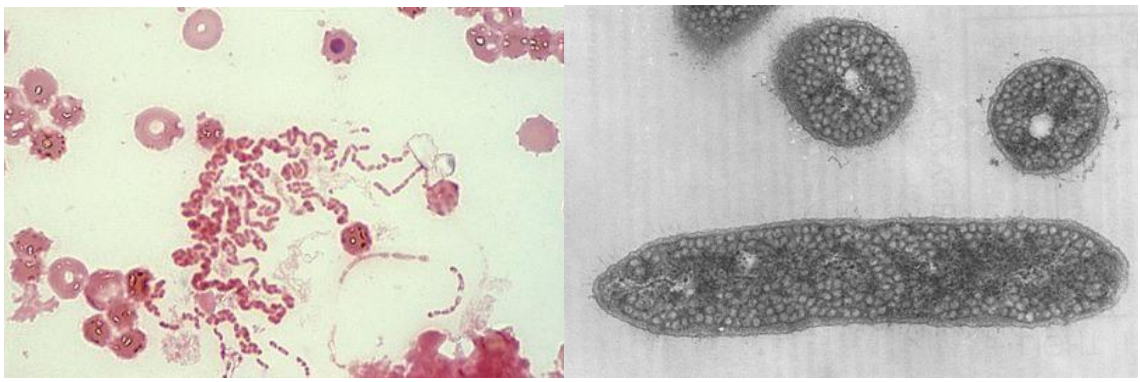


Figure 1.5: Microscopic images of *R. capsulatus* a) (Kenyon College) b) (The Institute of Molecular Biology and Biotechnology)

Rhodopseudomonas palustris

Rhodopseudomonas palustris is a rod-shaped gram-negative purple non-sulfur bacteria strain, from *Bradyrhizobiacecae* family and *Rhodopseudomonas* genus. It is commonly found in soils and water. It multiplies by budding (Whittenbury and McLee., 1967). Under anaerobic conditons and in the presence of light, thylakoids, which are intracytoplasmic membrane vesicle systems, and photosynthetic reaction centers form (U.S. Department of Energy, Lang and Oesterhelt, 1989). *R. palustris* also includes bacteriochlorophyll *b* at the center of the photosynthetic reaction center (Lang and Oesterhelt, 1989). *R. palustris* can accumulate biomass by carbon dioxide absorption, however can also utilize aromatic compounds constituting lignin, and even toxic compounds such as 3-chlorobenzoateto (U.S. Department of Energy). It can use dimethyl sulfoxide, potassium nitrate, or sodium nitrite as electron acceptor, intead of oxygen (Lang and Oesterhelt, 1989). It also has a potential to be used as a microorganism to remove pollutants from illuminated anaerobic habitats like lakes,

waste lagoons, sediments of ditches and ponds, mud, and moise soil (McGrath and Harfoot, 1997). Absorption spectra of *R. palustris* have maxima at 370-375, 585-589, 802, and 850-856 nm (Gall and Robert, 1999). Microscopic images of *R. palustris* are given in Figure 1.6.

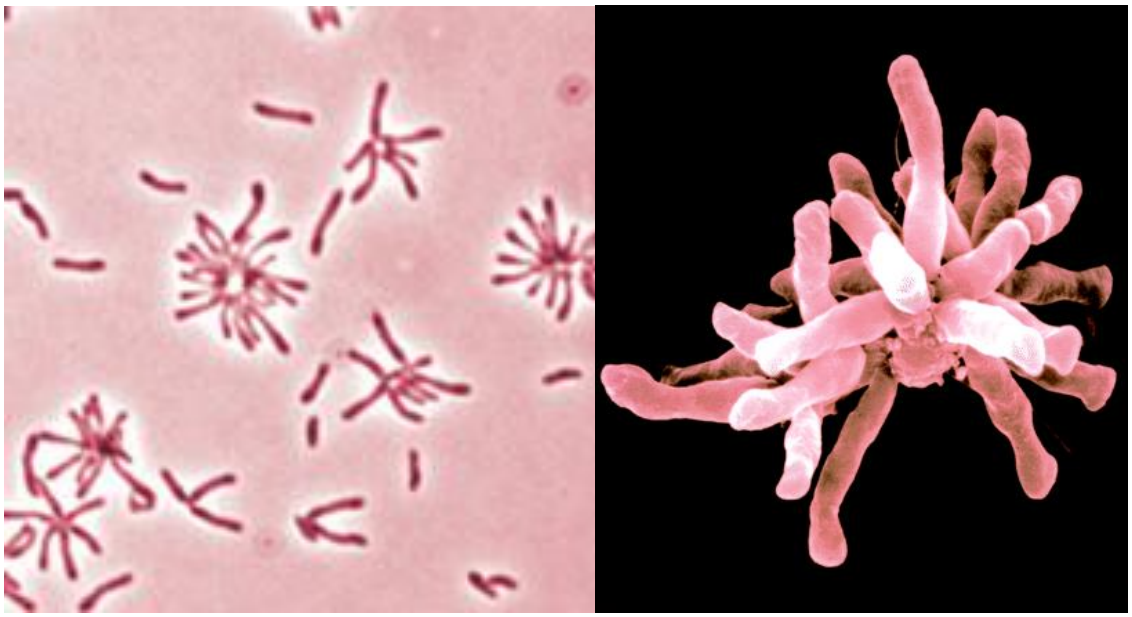


Figure 1.6: Microscopic images of *R. palustris* a) (U.S. Department of Energy) b) (American Society for Microbiology)

Rhodobacter sphaeroides

Formerly classified as a *Rhodopseudomonas* species, *Rhodobacter sphaeroides* is currently classified under *Rhodopseudomonas* genus. *R. sphaeroides* can be found in many shapes including heart shaped and short rods, however it is usually found in ovoid shape. *R. sphaeroides* cells may occur in pairs, which might be connected by a slender

filament or tube (Pellerin and Gest, 1983). It can exhibit dirty greenish brown to dark brown color, however in the presence of oxygen, color turns to red (Holt et al., 1984). Its width is usually found to be 0.5 µm and its legth is usually found to be between 2 and 2.5 µm, however, cells grown on sugar containing media may enlarge up to 3-3.5 µm in diameter (Pellerin and Gest, 1983).

Fresh cultures usually have low density and exhibit transiet flocculation. However with time, they exhibit slime formation and become viscous. Slime formation is observed in cultures after incubation for several days, and is enhanced in compex media (Pellerin and Gest, 1983). Polar flagella provides motility to especially young cells, however motility is ceased in alkaline environments (Holt et al., 1994).

R. sphaeroides contain bacteriochlorophyll *a* and carotenoids of spheirodene series, which have maxima of absorption spectra at 372-375, 414-416, 446-450, 474-481, 507-508, 586-588, 800-805, 850-852 and 870-875 nm (Pellegrin and Gest, 1983). Niacin, thiamine and biotin are required vitamins as growth factors for *R. sphaeroides* species (Biebl and Pfenning, 1981). Microscopic image of *R. sphaeroides* is given in Figure 1.7.

Figure 1.7: Microscopic image of *R. sphaeroides* (Varga and Kaplan)

1.4.2 Mechanisms of Hydrogen Production in PNS Bacteria

Hydrogen production by PNS bacteria requires an illuminated and anaerobic environment, and organic substrates. Hydrogenase and nitrogenase enzymes are the main enzymes involved in hydrogen metabolism, which are complemented with TCA cycle and photosynthetic membrane apparatus (Koku, 2002).

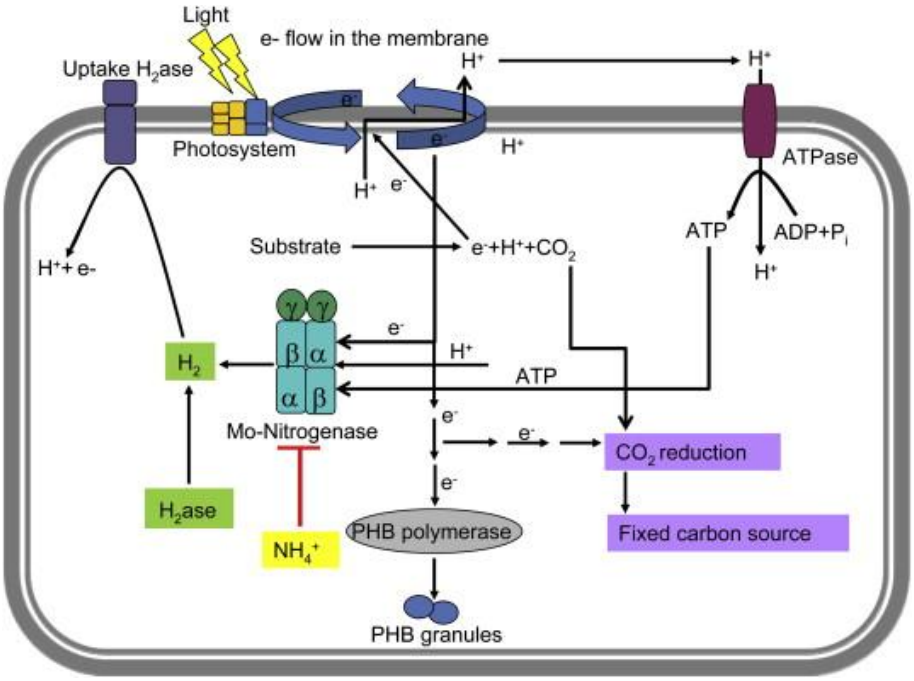


Figure 1.8: Photofermentation by PNS bacteria (Kars and Gündüz, 2010)

The starting point of the mechanism is the TCA cycle, releasing electrons, protons and carbon dioxide from oxidation of organic substrates. In the next step, the electrons and protons which are released from the TCA cycle are diverted to the nitrogenase enzyme via oxidation and reduction reactions by carriers NAD^+ (Nicotinamide adenine dinucleotide) and Fd (Ferrodoxin) (Vignais et al., 1985). Also the ATPs produced by the photosynthetic membrane apparatus by photophosphorylation are diverted to the nitrogenase enzyme. The utilized protons are supplied from the TCA cycle and ATP-synthase which also belongs to the photosynthetic membrane apparatus. Nitrogenase enzyme produces hydrogen molecules by reduction of protons (Sasikala et al., 1990). (Figure 1.8)

On the other hand, hydrogenase enzyme converts molecular hydrogen to protons and electrons, hence has a role of an uptake enzyme. As a result, the net molecular hydrogen production is the difference between the amount of molecular hydrogen produced by the nitrogenase and the amount of molecular hydrogen consumed by the hydrogenase enzymes (Vignais et al., 1985).

1.4.3 Enzymes Involved in Hydrogen Production

Hydrogen production by PNS bacteria is governed by hydrogenase and nitrogenase enzymes.

Hydrogenase

Hydrogenase enzyme catalyzes of the oxidation of molecular hydrogen, which is reversible as shown below in Equation 1.11:

$$H_2 \leftrightarrow 2H^+ + 2e^- \tag{1.11}$$

Hydrogenases depending on the active site of the enzymes are classified as, Fe-only hydrogenases, Fe-Fe hydrogenases and Ni-Fe hydrogenases depending on the metal cofactor present in the active site.

(i) [Fe]-hydrogenases, discovered in archea *Methanothermobacter marburgensis*, were initially called metal-free hydrogenases, however later renamed as iron-sulfur cluster-free hydrogenases. They have a mononuclear Fe active site and are devoid of iron-sulfur clusters. Fe

active sites are coordinated with two CO-, one sulfur- and one or two N/O ligands. Their open sites are proposed to be binding location for hydrogen (Shima and Thauer, 2007, Korbas et al., 2006). [Fe]-hydrogenases does not only differ from [FeFe] and [NiFe] hydrogenases by structure, but also by the Fe required for enzyme activity not being redox active. They have different catalytic activities than other hydrogenases, by catalyzing an intermediary step in carbon dioxide reduction with hydrogen to methane, instead of $2H^+ + 2e^- \rightarrow H_2$ (Vignais and Billoud, 2007).

(ii) [Fe-Fe]-hydrogenases are found in green algae, strict anaerobes, fungi and protiststt. They are mostly monomeric; however ones with two, three or four subunits are also reported in the literature. They contain only Fe and S in their catalytic site and are involved in hydrogen production rather than oxidation. Fe atoms of the [4Fe-4S] cluster are coordinated by cysteine residues. 2 Fe centers whereas, are coordinated by CO and CN ligands. (Ghirardi et al., 2007).

(iii) [Ni-Fe]-hydrogenases, found in cyanobacteria, have two functionally different types. One type is uptake hydrogenases, which recycles H_2 produced from nitrogenase, mostly in nitrogen fixing bacteria. The large catalytic subunit has Ni- and Fe- binding sites at the N- and C- terminals. The small subunit has eight cysteine residues, which are probably involved in [FeS] cluster formation. The other type is bi-directional hydrogenases, which recycle or produce hydrogen, depending on the physiological requirements. Bi-directional hydrogenases have cysteine residues involved in binding Ni and Fe to active sites at the large subunit.

The small subunit possesses cysteine residues, probably involved in coordination of [4Fe-4S] cluster, similarly to the small subunit of the uptake hydrogenase. A third subunit is thought to be involved in membrane attachment and electron transport, as a bridging subunit (Ghirardi et al., 2007).

Bi-directional hydrogenase catalyzes consumption of hydrogen molecules if hydrogen molecules and electron acceptors are present. On the other hand, if electron donors with low ionization potential are present, the enzyme catalyzes formation of hydrogen molecules (Vignais et al., 1985). Oxygen and carbon monoxide inhibits hydrogenase enzyme (Sánchez et al., 2010).

Deletion of uptake hydrogenase enzyme has found to increase hydrogen yield (Kars, 2008, Öztürk et al., 2006). Uptake hydrogenase enzyme was deleted via site directed mutagenesis by Kars et al., (2008) to produce a hup- strain of *R. sphaeroides* O.U.001 and via interposon mutagenesis by Öztürk et al., (2006) to produce a hup- strain of *R. capsulatus* MT1131, which was named *R. capsulatus* YO3. Both mutants have exhibited better hydrogen production profiles when compared with the wild types of the same strains (Kars, 2008, Öztürk et al., 2006).

Nitrogenase

The main enzyme responsible for the hydrogen production capability of the PNS bacteria is the nitrogenase enzyme. The main function of the nitrogenase enzyme is dinitrogen fixation by reduction of dinitrogen to ammonia (Burns et al., 1975). The reaction is shown below in Equation (1.12):

$$N_2 + 8\ H^+ + 8\ e^- + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i \qquad (1.12)$$

However, in the absence of dinitrogen, enzyme catalyzes the formation of H_2 in anaerobic condition and under limited nitrogen source (Equation 1.13):

$$2\ H^+ + 2\ e^- + 4\ ATP \rightarrow H_2 + 4\ ADP + 4\ P_i \qquad (1.13)$$

There are three types of nitrogenases according to their active site elements:

i) Fe-Nitrogenase (Iron centered)
ii) V-Nitrogenase (Vanadium centered)
iii) Mo-Nitrogenase (Molybdenum centered)

Nitrogenases of the PNS bacteria are generally found out to be molybdenum type nitrogenases (Koku, 2001). However, *R. capsulatus* involves both molybdenum and iron nitrogenases (Masepohl and Hallenbeck, 2010).

Oxygen inhibits nitrogenase activity and leads to up to three times less hydrogen production when compared with experimentations in anaerobic conditions (Akköse, 2008). High ammonium concentrations were also found to repress the activity of the nitrogenase enzyme (Fu and Burris, 1989, Pierrard e. al., 1993).

1.5 Improvement of Hydrogen Production by PNS Bacteria

Although PNS bacteria are known with their hydrogen production capability,

improvement of hydrogen yield and efficiency is necessary for large scale outdoor H_2 production. Routes to increase the efficiency of the hydrogen production process are, i) immobilization of the PNS bacteria, ii) improvements over reactor design, iii) genetic modifications, and iv) co-cultivation of different PNS bacteria. Additionally different concentrations of ammonium source, acetate and aerobic conditions influence hydrogen yield and productivity by regulation of nitrogenase (Akköse et al., 2009).

1.5.1 Immobilization

Immobilization is the confinement or localization of cells to a specific region of space in order to preserve some desired catalytic activity (Karel et al., 1985). Immobilization of PNS bacteria is one of the procedures which can be performed for increased hydrogen yield (Basak and Das, 2007).

The advantages of the immobilization technology are as the following: i) high cell concentrations are maintained, ii) cells are re-used, hence, cell recovery and recycling costs are eliminated, iii) cell wash-out problems are eliminated for continuous cultures, iv) high cell concentrations and flow rates result in high volumetric productivities, v) favorable micro-environmental conditions can be provided, vi) genetic stability can be improved, and vii) shear damages due to mixing or aeration of the reactor are eliminated. However, the following are the main limitations of the immobilization technology: i) product might be required to be extracted from the cells, ii) complications may arise due to diffusion limitations, iii) due to heterogeneity, it might be difficult to control the microenvironment, and iv) growth and evolved gas might cause disruption to the immobilized matrix (Elkahlout, 2011).

Immobilization on glass surfaces, reverse micelle microeactors which have hydrophilic cores and hydrophobic groups extending away, and agar are among experimented practices (Tsygankov et al., 1993, Singh and Misra, 2009, Elkahlout, 2011). Utilization of reverse micelle reactors has leaded 50 fold increased production rate for *R. palustris* (Singh and Misra, 2009). For agar immobilized systems, 3 and 4 % agar concentrations have resulted in the most acceptable results for *R. capsulatus* DSM 1710 and YO3. Higher agar concentrations were reported to cause diffusion limitations for nutrients and products. Also higher nitrogen source was requires since 4 mM glutamate has yielded better results than 2 mM glutamate and 60 mM Acetate has resulted in the highest yields. Maximally 3.4, 3.4, 2.4 mmol H_2 / mmol acetate and 2.3, 3.1 and 3 mmol H_2 / mmol acetate substrate yields were observed for *R. capsulatus* YO3 and DSM 1710 respectively. Additionally agar immobilized systems has leaded 1.14-1.41 folds increased productivity for co-immobilization with *H.salinarium* (Elkahlout, 2011)

1.5.2 Reactor Design

Reactor type is an important parameter for hydrogen yield and productivity. Photobioreactors shall conduct the light to the cells and keep cells, substrates and products enclosed and uncontaminated (Tredici, 2004). The important thing in reactor design is high light availability and optimal area to volume ratio. Hence light energy can penetrate through cells better can be utilized maximally per volume of culture and better distribution of light over the reactor volume is maintained (Akkerman et al., 2002). Thin surfaces would be sensitive to shocks but would allow high light penetrance. On the contrary thick surfaces would be more resilient; however more expensive and less light would be penetrated through them. High surface area to volume ratios would result in better light distribution. However high surface areas to volume ratios require smaller

reactor sizes, hence the lightened area to cultured media ratio would remain low and the solar would not be benefited completely, since some of the penetrated light would exit the photobioreactor. On the other hand, very small surface area to volume ratios result in limited light penetration zones, hence leaving an important ratio of the culture unilluminated. High cell concentrations also cause limited light penetration zones and result in light inhibition, light saturation, light limitation, which all may co-exist within the same reactor setup. Bidirectional mixing is a solution for such phenomenon by moving cells between different zones (Ogbanna and Tanaka, 2001).

There are various reactor types, such as: i) panel photobioreactors, tubular photobioreactors which are utilized for photofermentative hydrogen production. Panel bioreactors are transparent, rectangular boxes having 1-5 cm depth (Akkerman et al., 2002). They are usually placed vertically, but they can be tiled at optimal degrees for maximal sun light exposure (Tredici and Zitelli, 1997, Richmond et al., 1999). Also flat reactors can be arranged in a stack close to each other, which allows and effect called lamination, five-fold dilution of the solar radiance, allowing higher efficiency of conversion into biomass (Carlozzi, 2000, Richmond and Zhang, 2001). The major drawback of panel photobioreactors is the lack of stirring systems. However bubbling gas can be used for mixing (Akkerman et al., 2003).

Tubular photobioreactors, on the other hand, consist of long transparent tubes with diameters varying between 3 and 6 cm. Tubular photobioreactors have higher surface area to volume ratio. Pumps are utilized for circulation of the cultures. Tubes can be installed through various ways, such as (Akkermann et al., 2002): i) horizontally as straight tubes with u-bends, ii) vertically by bending as cylinder or cone coils, iii) vertically as fence-like structures with u-bends or connecting manifolds, iv) horizontal

parallel tubes connected by manifolds.

Hydrogen production by photosynthetic bacteria has been experimented in tubular and panel photobioreactors operated in continuous mode (Sarı, 2007, Uyar, 2008, Androga 2009, Boran 2011). 0.009LH_2/L_c.h hydrogen production rate was reported by Sarı (2007) using helical tubular bioreactors with *R. sphaeroides* O.U. 001. Uyar (2008), on the other hand has reported 0.52 and 0.27 mg/L_c.h hydrogen production rates using panel bioreactors with *R. capsulatus* (hup-) YO3 and DSM 1710 respectively. Androga (2009) has obtained 0.4 mmol H_2 / L_c.h using panel bioreactors with *R. capsulatus* (hup-) YO3. Boran (2011) has obtained 0.2 and 0.3 mmol H_2 / m^3.h using tubular bioreactors with *R. capsulatus* (hup-) YO3 and DSM 1710 respectively.

1.5.3 Genetic Modifications

Hydrogen yield and productivity can be increased by various genetic manipulations including deletion of uptake hydrogenase, modification of ammonium regulation mechanism or PHB synthesis. Uptake hydrogenase (Hup) is an enzyme, which consumes hydrogen. Hup- mutants of *R. sphaeroides* O.U.001 and *R. capsulatus* MT1131 were produced by Kars et al. (2008) via site directed mutagenesis and by Öztürk et al. (2006) via interposon mutagenesis respectively. Hup- mutants of these strains have exhibited higher hydrogen yield. While *R. capsulatus* MT1131 has resulted in 0.010 mL/mL_c.h hydrogen production and 24 % substrate conversion efficiency, *R. capsulatus* YO3 has resulted in 0.019 mL/mL_c.h hydrogen production and 37 % substrate conversion efficiency (Öztürk, 2006). The hup- mutant of *R. capsulatus* MT1131, which was named *R. capsulatus* YO3 had been one of the bacterial strains experimented in co-cultivation setups throughout this study.

One other genetical modification increasing hydrogen yield is on ammonium regulation. Ammonia is the feedback inhibitor of nitrogenase. Also, hydrogen production is favoured under nitrogen limited conditions by PNS bacteria. Drepper et al. (2003) have produced glnB-glnK double knockout mutant of *R. capsulatus*, which was able to synthesize active molybdenum nitrogenase even under environments containing high ammonium concentrations. By this modification, they could surpass the ammonium inhibition and have increased the hydrogen yield. While 928±155 and 0±0 nmol H_2 / mg.h was reported for nitrogen limited and nitrogen containing environments respectively with wild type *R. capsulatus* B10S strain, for glnB-glnK double knockout mutant *R. capsulatus* TD116, these results were increased to 1431±143 and 1277±2380 nmol H_2 / mg.h respectively.

PHB is a side product of hydrogen production by PNS bacteria. PHB synthesis pathway competes for electrons and reduces the hydrogen yield. Switching off the PHB synthase is a genetic modification for improving hydrogen productivity. Kim et al. (2006) have deleted PHB synthase from *R. sphaeroides* KD131 and obtained higher hydrogen yields. 1.32 and 3.34 ml H_2/mg-dcw hydrogen production rates were reported for the wild-type and Hup-/Phb- mutant strain. Elimination of cyt cbb3 oxidase on the other hand, which serves as a redox signal to RegB/RegA regulatory system, increases the number of nitrogenase expression, leads to increase in hydrogen production 2-fold (Öztürk et al., 2006). There are some other genetic modifications for example, reducing the antenna size to improve light utilization which minimizes the absorbance of pigments in the first layer, hence ameliorate the light distribution and permits better light transmittance (Melis et al., 1998), recombinant expression of H_2 producing hydrogenases, etc.

1.5.4 Co-culture

Co-cultivation method is a promising alternative for the improvement of hydrogen production. Type of the bacterial strain is one of the most important parameters that influence the photofermentation process. Different PNS bacteria are known to exhibit different hydrogen production performances on different substrate types. Determination of co-culture combinations which exhibit high hydrogen yields and productivities therefore can be useful for obtaining high hydrogen efficieny from an array of different substrate types and substrate mixtures. Also co-cultivation of PNS bacteria with different light absorption spectra might result in better utilization of light. Also different strains have different sensitivity to physical conditions such as pH, temperature, and different substances can be inhibitory on different bacteria. In a co-cultivation setup, when some undesired conditions are present for some strain affecting hydrogen production, the other strain(s) might not be affected these conditions and vice versa. There have been various studies investigating the benefits of co-utilization.

In one study, hydrogen production potential of co-cultivation of *R. sphaeroides* O.U.001 (ABP), *Synechococcus cedrorum* (BGA) and *Pseudomonas fluorescens* (HET) strains have been investigated (Sasikala and Ramana, 1994). They have observed various results with co-cultivated combinations of these bacteria, reaching up to 3.87 fold increased hydrogen yield for the co-culture including all three strains on an immobilized setup. Hence the synergistic effects of multiple bacteria in co-culture setups on hydrogen production can be beneficial for increasing hydrogen yield and productivity on a wider set of substrates and substrate mixtures.

Co-cultures of Purple Non-Sulfur Bacteria

In one study, the effects of co-cultivation of *Rhodopseudomonas* TUT 3630 and *Rhodobacter* TUT 3733 strains has been investigated. Mixed populations have been grown in semi-aerobic conditions under light, and with different acetate concentrations, and bacterial populations have been quantified by 16S rRNA targeted FISH and PCR-denatured gradient gel electrophoresis (Okubo et al., 2007). They have concluded that, acetate concentrations in the feed ranging from 5 mM to 20 mM results in dominance by *Rhodobacter* species, whereas less than 1 mM acetate in the feed results in dominance by *Rhodopseudomonas* species. They suggest that acetate concentration is one of the key parameters determining the dominance of different bacterial species by claiming that *Rhodobacter* species having low affinity, whereas *Rhodopseudomonas* species having high affinity for acetate. They explain this might be the reason of *Rhodobacter* species being the dominant species of wastewaters containing high levels of lower fatty acids, whereas *Rhodopseudomonas* species dominate the wastewaters containing low levels of lower fatty acids (Okubo et al., 2007). Hence even some co-cultivation combinations may not be suggested to improve hydrogen yield on certain conditions such as a certain acetate concentrations, the ability to produce hydrogen, may in fact be widened to a wider range of acetate concentrations.

Sasikala et al., (1994) have co-cultivated free and immobilized forms of *R. sphaeroides* O.U. 001 and Pseudomonas *fluorescens* on BP medium with 1% (w/v) glucose addition. Upon comparison of these co-cultures with single cultures of the same strains, nearly 4-fold increase in comparison with single culture *R. Sphaeroides*, and more than 26-fold increase in comparison with *Pseudomonas fluorescens* were observed.

H_2 production performances of co-cultivated PNS bacteria on sugar beet molasses have been the subject of another study (Sağır, 2012). Sağır has investigated photofermentative hydrogen production by PNS bacteria on molasses medium with 5 mM sucrose, and have compared the results with the single cultures of the same bacteria. Sağır has observed increased hydrogen production when compared to single cultures, up to 2.37 fold when compared with the strains resulting with higher hydrogen yields in single culture setup, and up to 2.86 fold when compared with the strains resulting with lower hydrogen yields in single culture setup. However, since different substrate ingredients result in different hydrogen yields and productivities, more experimentations of co-cultivation of PNS bacteria are required.

1.6 Aim of the Study

The main objective of this study is to investigate hydrogen production efficiency of different co-cultures of PNS bacteria on defined medium.

For this purpose, growth, hydrogen production and substrate utilization of single cultures and co-cultures of different PNS bacteria (*R. capsulatus* (DSM 1710), *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864)) were compared on defined BP medium containing 40 mM Acetate / 2 mM Glutamate in 150 mL photobioreactor under continuous illumination and anaerobic conditions.

This study is based on defined culture media, and hence will constitute a standard for previous and forthcoming studies related to co-cultivation of *R. capsulatus* hup-, *R. capsulatus*, *R. palustris* and *R. sphaeroides* on variety of different wastes / waste waters.

CHAPTER 2

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Bacterial Strains

During this study, four different bacterial strains were used: *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127), *R. sphaeroides* O.U.001 (DSM 5864), and *R. capsulatus* YO3 hup-. Among these bacterial strains; *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) were obtained from DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH* – German Collection of Microorganisms and Cell Cultures, Braunsweig Germany), and *R. capsulatus* YO3 hup-, which is a mutant strain of *R. capsulatus* MT1131 lacking uptake hydrogenase enzyme, was prepared by Öztürk et al. (2006) (Table 2.1).

Table 2.1: Classification of PNS bacteria used in this study

Domain	Bacteria	Bacteria	Bacteria
Phylum	Proteobacteria	Proteobacteria	Proteobacteria
Class	Alphaproteobacteria	Alphaproteobacteria	Alphaproteobacteria
Order	Rhodobacterales	Rhizobiales	Rhodobacterales
Family	Rhodobacteraceae	Bradrhizobiacae	Rhodobacteraceae
Genus	*Rhodobacter*	*Rhodopseudomonas*	*Rhodobacter*
Species	*Capsulatus*	*palustris*	*sphaeroides*

2.1.2 Chemicals

Among the chemicals used for experiments, Potassium dihydrogen phosphate, magnesium sulfate heptahydrate, Calcium chloride dihydrate, Monosodium glutamate, Acetate, Iron-citrate, Trace elements, Bacto pepton, Magnesium chloride, and Difco-bacto agar were obtained from MERCK Chemicals, Thiamine, Niacin and Biotin were obtained from SIGMA Chemicals and Yeast extract was obtained from OXOID Chemicals.

2.2 Methods

2.2.1 Culture Media

In this study, bacteria were cultivated in different media depending on the purpose of utilization. Standard BP media (*Biebl* and *Pfenning*, 1981) (Appendix A) with acetate and glutamate as carbon and nitrogen source respectively were used as liquid media in two different concentrations for acetate and glutamate, named Growth Medium and Hydrogen Production Medium. Solid MPYE medium was used to check the contamination and activation from -80 °C stocks.

Growth Medium

Standard BP medium (*Biebl* and *Pfenning*, 1981) with 20 mM acetate and 10 mM glutamate addition as carbon and nitrogen sources, respectively, was used for the activation of bacteria. Bacteria were grown in growth medium before inoculation to hydrogen production medium.

The growth medium contained (for 1 L): 3 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.05 g $CaCl_2.2H_2O$, 1.15 g Na-glutamate, and 1.85 mL acetate were added to approximately 900 mL of distilled water and mixed throughly. Then the pH was adjusted to 6.3-6.4 with HCl / KOH, and the total volume was completed to 1000 mL with distilled water. After autoclaving for 30 min. at 121°C, 1.06 Bar, medium was cooled to 24°C and 0.1 mL of vitamin solution, 0.5 mL Fe-citrate solution, and 0.1 mL trace elements solution were added, and mixed thoroughly. Preparations of vitamin solution, trace elements and Fe-citrate solution are given in Appendix (A).

Hydrogen Production Medium

Standard BP medium (*Biebl* and *Pfenning*, 1981) with 40 mM acetate and 2 mM glutamate addition as carbon and nitrogen sources, respectively, used for H_2 production. Hydrogen production medium contained (for 1L): 3 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.05 g $CaCl_2.2H_2O$, 0.36 g Na-glutamate, and 2.29 mL acetate were added to approximately 900 mL of distilled water and mixed throughly. Then the pH was adjusted to 6.3-6.4 with HCl / KOH, and the total volume was completed to 1000 mL with distilled water. After autoclaving for 30 min. at 121°C, 1.06 Bar, medium was cooled to 24°C and 0.1 mL of vitamin solution, 0.5 mL Fe-citrate solution, and 0.1 mL trace elements solution were added, and mixed thoroughly. Preparations of vitamin solution and trace elements solution are given in Appendix (A).

MPYE Medium

Solid MPYE media were used in order to test whether the bacteria were contaminated. Bacteria were inoculated and spread to MPYE plates using a loop. MPYE plates were prepared as explained below:

For preparation of the MPYE plates, 3 g Bacto pepton (0.3%), 3 g Yeast extract (0.3%), 1.6 mL 1 M $MgCl_2$, and 1 mL 1 M $CaCl_2$ were dissolved in 1000 mL distilled water. Then the pH was adjusted to 7, and finally 15 g (1.5 %) Difco-Bacto agar was added, and the mixture was autoclaved. After autoclave, media were shaken continuously and cooled down to 30°C before pouring into plates.

2.2.2 Activation of Bacteria from -80 °C Glycerol Stocks

Stock cultures were taken from -80°C for *R. capsulatus* (DSM 1710), *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864). From vials of bacteria which were taken from -80°C, spreading was done on MPYE agar plates in order to see whether the cultures were clean or contaminated. The bacteria inside the vials were separated into two eppendorfs for each strain with a volume of 0.5 mL which was complemented with 1 mL growth medium for each eppendorf. Cultures were exposed to 2000 lux light intensity. Contaminations were not observed in plates. Bacteria grown in two eppendorfs for each strain were transferred to a 15 mL falcon tubes and completed to 15 mL by 12 mL growth medium. Cultures were exposed to 2000 lux light intensity by illumination with 100 W tungsten lamps. Bacteria were transferred to 55 mL glass bottles and 35 mL growth medium was added for each bottle for photofermentation in batch mode. Cultures were exposed to 2000 lux by illumination with 100 W tungsten lamps.

2.2.3 Preparation of -80 °C Bacterial Glycerol Stocks

0.5 mL of bacterial cultures was transferred to sterile eppendorf tubes. 0.5 of mL sterile 60 % (v/v) glycerol solution was added to each eppendorf. Stocks were freezed and stored at -80 °C.

2.2.4 Activation of Bacteria

Passaging of bacteria grown in 55 mL glass bottles obtained from previous step to growth medium has been repeated several times to ensure that bacteria were active and

healthy. For physiological experiments on growth medium, bacterial inoculation (10 %) from freshly grown cultures were carried out into growth medium, when OD_{660} reaches 2.0. Growth, hydrogen and pH values were followed. Reactor volume was 55 mL. Light intensity was set to 2000lux.

2.2.5 Experimental Setup for Hydrogen Production

When absorbances of bacteria at 660 nm in growth medium reached to 2.0, each bacterial strain was inoculated to new bottles with a volumetric ratio of %10. Reactor volume was 150 mL. Bottles were filled with the H_2 production media and the bacterial cultures. Light intensity was set to 2000lux. pH, OD, Gas volume measurements, Gas Chromotography analyses were performed. Liquid samples at each day were taken to follow organic acid utilization by HPLC.

For single cultures, 10 % bacterial inoculum was used. Hence 15 mL of freshly grown bacteria at OD_{660} of 2.0 was inoculated to 135 mL of hydrogen production medium.

For co-cultures of two different PNS bacteria strains, 10 % + 10 % bacterial inoculums for both strains were used. Hence 15 mL of each bacterial strain grown in growth medium until absorbance value at 660 nm reaches 2 were inoculated to 120 mL of hydrogen production medium.

For co-cultures of three different PNS bacteria strains, 10 % + 10 % + 10 % bacterial innoculums for all three strains were used. Hence 15 mL of each bacterial strain grown in growth medium until absorbance value at 660 nm reaches 2 were inoculated to 105 mL of hydrogen production medium.

All cultures and media were transferred to the bottles inside sterile cabinet following UV sterilization for 15 minutes. All bottles were closed with sterile caps, and sealed with parafilm. All bottles and caps were sterilized via autoclaving prior to usage. Remaining airs inside the bottles were replaced with argon after capping, in order to maintain an anaerobic environment. Photobioreactors were placed inside a cooling incubator (Nüve, ES250) and illuminated by 100 W tungsten lamps. Temperature of the incubator was set to 30°C. Light intensities of the photobioreactors were set to within range of 2000-2080 lux by using a luxmeter (Lutron LX – 105 Light Meter). Evolving gas from each photobioreactor was measured volumetrically. Plastic tubing having syringe needles on both ends was used in order to transfer evolving gas from photobioreactors to volumetric gas collection colons. Volumetric gas collection colons were initially filled with distilled water, and re-filled upon depletion. (Figures 2.1 and 2.2)

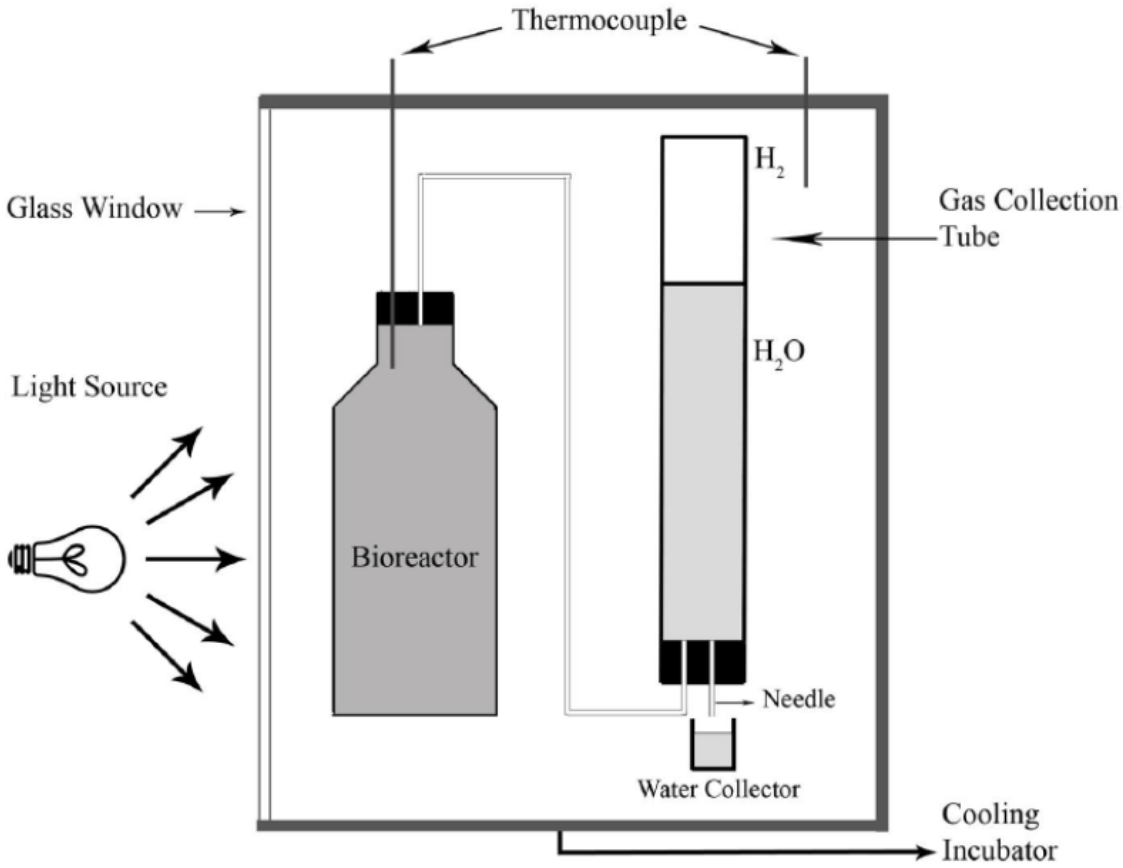


Figure 2.1: Schematic representation of hydrogen production setup (Sevinç, 2010)

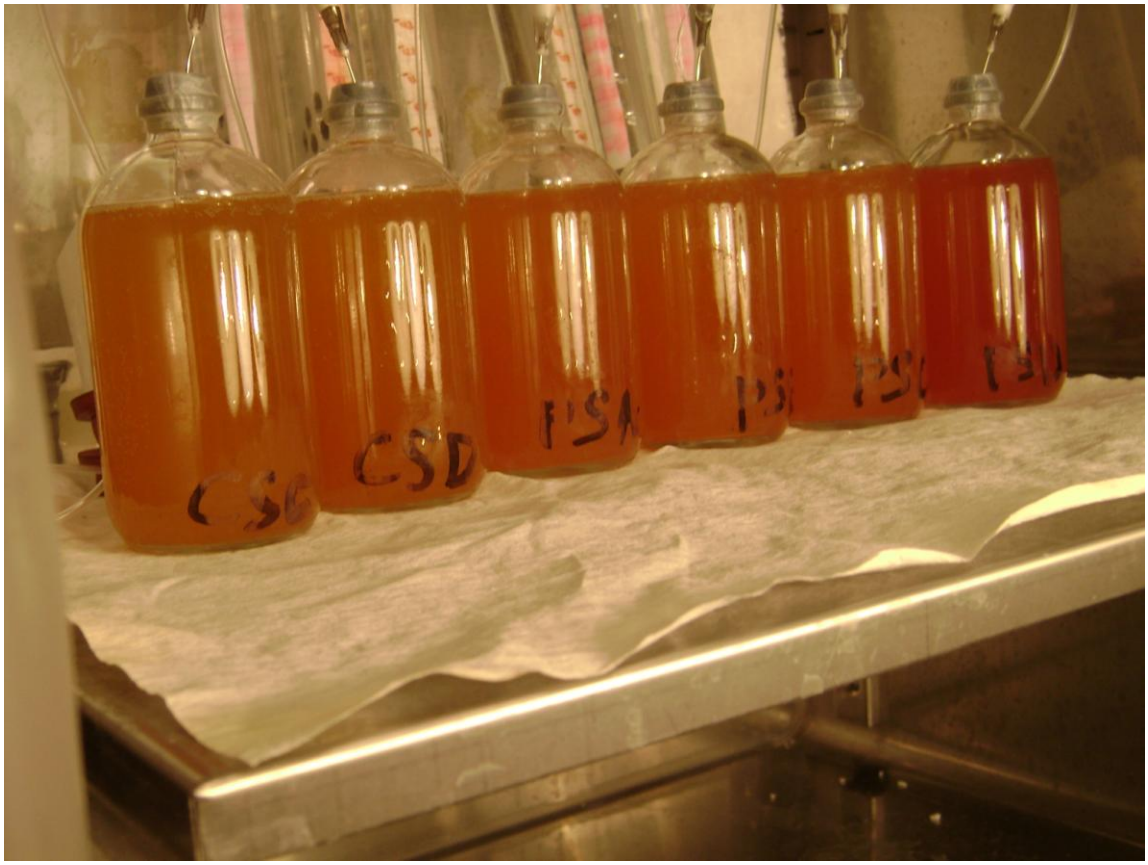


Figure 2.2: Experimental setup for co-cultivation of PNS bacteria

2.2.6 Measurements

At every 24th hour, 2.2 mL of liquid samples were taken for pH, OD measurements and organic acid quantifications via High Pressure Liquid Chromatography (HPLC). pH and OD measurements were done immediately, whereas samples for organic acid determinations were prepared by centrifugation of the samples at 13400 rpm in eppendorf minispin for 10 minutes for single cultures and for 20 minutes for co-cultures

of two and three different strains of PNS bacteria. The supernatant portion of each sample was transferred to a new eppendorf, and then stored at -20°C until HPLC analyses. Daily produced gas amounts were read from volumetric gas collection columns. Compositions of the produced gases were determined via Agilent Technologies 6890N Gas Chromatography by using 100 µL gas injections.

2.2.6.1 Cell Concentration Measurements

Cell concentration measurements were done via optical density measurements at 660 nm wavelength. An UV Spectrophotometer (Shimadzu UV – 1800) was used for optical density measurements. During OD measurements, distilled water was used as blank. Dry cell weights were estimated by using calibration curves (Uyar, 2008, Öztürk 2008), which have been included in Appendix (B).

2.2.6.2 pH Measurements

pH meaurements were performed by using a pH meter (Mettler Toledo 3311). The pH meter was calibrated with standard solutions of the manufacturer having pH values of 4.0, 7.0, and 9.21 prior to measurements.

2.2.6.3 Organic Acid Analysis

Liquid samples of 1.5 mL volume were collected at analysis intervals and centrifuged for 10 minutes for single cultures, and 20 minutes for co-cultures of two and three different strains of PNS bacteria at 13400 rpm speed in eppendorf minispin. Supernatants were transferred to new eppendorf tubes and stored at -20 °C until they

were used for HPLC analysis. Samples were defrosted and filtered through 45 µm nylon filters (Millipore, 13 mm) prior to HPLC analysis.

Shimadzu LC-20A Prominence HPLC system was used for acetate analysis. Alltech IOA-1000 (300 mm x 7.8 mm) HPLC caolumn was utilized in order to separate organic acids. Samples were loaded to autosampler (Shimadzu SIL-20AC). Organic acid determination was performed via an UV-VIS detector (Shimadzu SPD-20AV) at 210 nm wavelength. Sample injection volume was set to 20 µL, oven temperature was set to 66°C and flow rate was set to 0.4 mL /min. 0.085 M H_2SO_4 solution was used as mobile phase. For plotting of the calibration curves, acetate solutions in different concentrations were prepared and run on HPLC system. Sample organic acid chromatograms and calibration curves have been included in Appendix (C).

2.2.6.4 Gas Composition Analysis

100 µL of evolved gas was taken from the photobioreactors and injected to Gas Chromatography device (Agilent Technologies 6890N) for gas composition analysis via a gas-tight syringe (Hamilton SampleLock Syringe, 500 µL volume, 22 GA needle size). Supelco Carboxen 1010 gas column, which is a "Porous Layer Open Tubular" (PLOT) column, was used for separation of gases. A thermal conductivity detector was also present in the system, in order to determine separated gases. The carrier gas utilized was argon, with a flow rate of 26 mL /min and oven, injector and detector temperatures were set to 140, 160, and 170 °C, respectively.

Calibration of the gas chromatography device was performed with H_2, CO_2, and air. Agilent Technologies Agilent Chemstation software Version B.01.01 was used. A sample

gas chromatogram has been included in Appendix (D).

2.2.7 Data Analysis and Calculations

The hydrogen yields, substrate conversion efficiencies, hydrogen productivities, and light conversion efficiencies were calculated for each run and the definitions of these parameters are given below:

The definition of hydrogen yield is given below in Equation (2.1):

$$Y_{H_2} = \frac{\text{Moles of hydrogen produced}}{\text{Moles of acetate utilized}} \qquad (2.1)$$

An example calculation of hydrogen yield was included in Appendix (E)

Theoretical hydrogen yields reach up to 4.0 for acetate, given 4 moles of hydrogen is evolved per mole of acetate (Equation 1.10).

Hydrogen yield shows the moles of hydrogen produced, in comparison with the moles of substrate utilized. Hence the comparison of hydrogen yields for different single cultures and co-cultures of PNS bacteria shows the efficiency of H_2 production. The definition of substrate conversion efficiency is given below in Equation (2.2):

$$\text{Substrate Conversion Efficiency} = \frac{\text{Number of moles of hydrogen produced}}{\text{Number of moles of hydrogen that would be produced from complete utilization of initial substrates}} \times 100 \qquad (2.2)$$

An example calculation of substrate conversion efficiency was included in Appendix (F)

Substrate conversion efficiency shows the efficiency of the conversion of the total initial substrate to hydrogen. It is the amount of hydrogen produced when compared with the hydrogen to be produced if the substrate is converted to hydrogen completely. Hence it shows how much of the initial substrates could be successfully utilized for production.

The definition of hydrogen productivity is given below in Equation (2.3):

$$\text{Hydrogen Productivity} = \frac{\text{Number of millimoles of hydrogen produced}}{\text{Volume of culture (L) x time (h) from the end of the lag phase to the end of the hydrogen production}} \qquad (2.3)$$

An example calculation of hydrogen productivity was included in Appendix (G)

Hydrogen productivity bears an industrial significance, since bacterial cultures which can produce maximum amount of hydrogen in minimum duration can be determined.

Maximum hydrogen productivities are calculated at an interval at which hydrogen is produced linearly.

The definition of light conversion efficiency is given below in Equation (2.4):

$$\eta\ (\%) = \frac{33.6 \times \rho_{H_2} \times V_{H_2}}{I \times A \times t} \times 100 \qquad (2.4)$$

Where 33.6 is the energy density of hydrogen gas (W.h / g), V_{H2} is the volume (L), ρ_{H2} is the density (g/L) of the produced hydrogen gas, I is the light intensity (W/m^2), A is the irradiated area (m^2), and t is the duration of hydrogen production from the end of the lag phase to the end of the hydrogen production (Özgür et al., 2010).

An example of calculation of light conversion efficiency was shown in Appendix (H)

The definition of specific growth rate is given below in Equation (2.5)

$$\mu = K' = \frac{\ln(m_{t_2}/m_{t_1})}{t_2 - t_1}; \quad t_2 > t_1 \tag{2.5}$$

Where m_{t1} is the biomass at time t_1, and m_{t2} is the biomass at time t_2.

Specific growth rate is the increase in cell mass per unit time during the period from the end of the lag phase to the end of the biomass growth.

Standard errors of means were calculated using at least three different samples for each calculation.

Standard error of the means were calculated for the sets of measurements and calculated data as given below in Equation (2.6)

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}} \tag{2.6}$$

Where s is the sample standard deviation and n is the sample size.

Sample standard deviation is calculated according to Equation (2.7)

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2}, \tag{2.7}$$

Where N is the sample size and the X_i is the ith sample.

CHAPTER 3

3. RESULTS AND DISCUSSION

Single cultures of PNS bacteria were tested on growth medium, and growth profiles, total hydrogen production values, and pH values were determined. From the growth profiles obtained, growth curves of PNS bacteria were deduced. Also single, and co-cultures of PNS bacteria in combinations of two and three different strains have been tested on hydrogen production medium, and growth profiles, total hydrogen production, pH values and acetate utilizations were determined, and hydrogen yields, substrate conversion efficiencies, hydrogen productivities, light conversion efficiencies and specific growth rates were calculated. Hence hydrogen production performances of single and co-cultures of PNS bacteria were compared.

3.1 Growth and Hydrogen Production by Single Cultures of PNS Bacteria

3.1.1 Growth, Hydrogen Production on Growth Medium

Experiments on growth medium have been carried out in order to adapt the bacteria to acetate medium and maintain their metabolic activity. Also the growth curves of PNS bacteria have been obtained from the results of the experiments carried out on growth medium. The growth medium used has included 20 mM acetate as carbon source and 10 mM glutamate as nitrogen source. This medium with high glutamate concentration has been used for maintaining high growth in biomass, since hydrogen production is inhibited by nitrogen, hence a metabolic activity biased towards biomass growth instead

of hydrogen production would be expected with such a medium composition. As given in Figure 3.1, *R. capsulatus* hup- (YO3) has exhibited instant growth; however *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) had a lag period of 24 hours, attributed to the adaption of bacteria on acetate. Total hydrogen production values have correlated with growth profiles (Figure 3.2): *R. capsulatus* hup- (YO3) has started H_2 production immediately (within 24 hours), whereas *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) have exhibited a lag period similar to the growth profiles. Total hydrogen production was observed the highest for *R. sphaeroides* O.U.001 (DSM 5864) with 0.56±0.03 L / L_c, which was followed by *R. capsulatus* hup- (YO3) with 0.51±0.02 L / L_c, and *R. palustris* (DSM 127) with 0.28±0.01 L / L_c. Although hydrogen production was not expected for bacteria cultured on growth medium with high glutamate concentration, hydrogen production was observed, which might be due to depletion of nitrogen source due to high growth rates. pH values have varied between 6.4-7.8 during growth (Figure 3.3). *R. capsulatus* hup- (YO3) has exhibited instant pH rise, however *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) have exhibited lag phases in pH rise, similar to growth and total H_2 production values. The buffer capacity of potassium phosphate buffer was not exceeded. Hydrogen productivities were calculated as 0.13±0.00, 0.10±0.01 and 0.14±0.01 mmol / L.h respectively for *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864). Hence, the rates of hydrogen production are comparable for all single cultures of PNS bacteria. Also rates of hydrogen production have correlated with maximum hydrogen production data. Specific growth rates were calculated as 0.092±0.001, 0.050±0.004 and 0.048±0.004 h^{-1} respectively for *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864). Hence the specific growth rate of *R. capsulatus* hup- (YO3) has doubled the specific growth rates of *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM

5864), which were comparable. On the other hand, maximum biomass concentrations were found 1.241±0.009, 0.742±0.007 and 1.683±0.016 gdcw / L_c for *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) respectively.

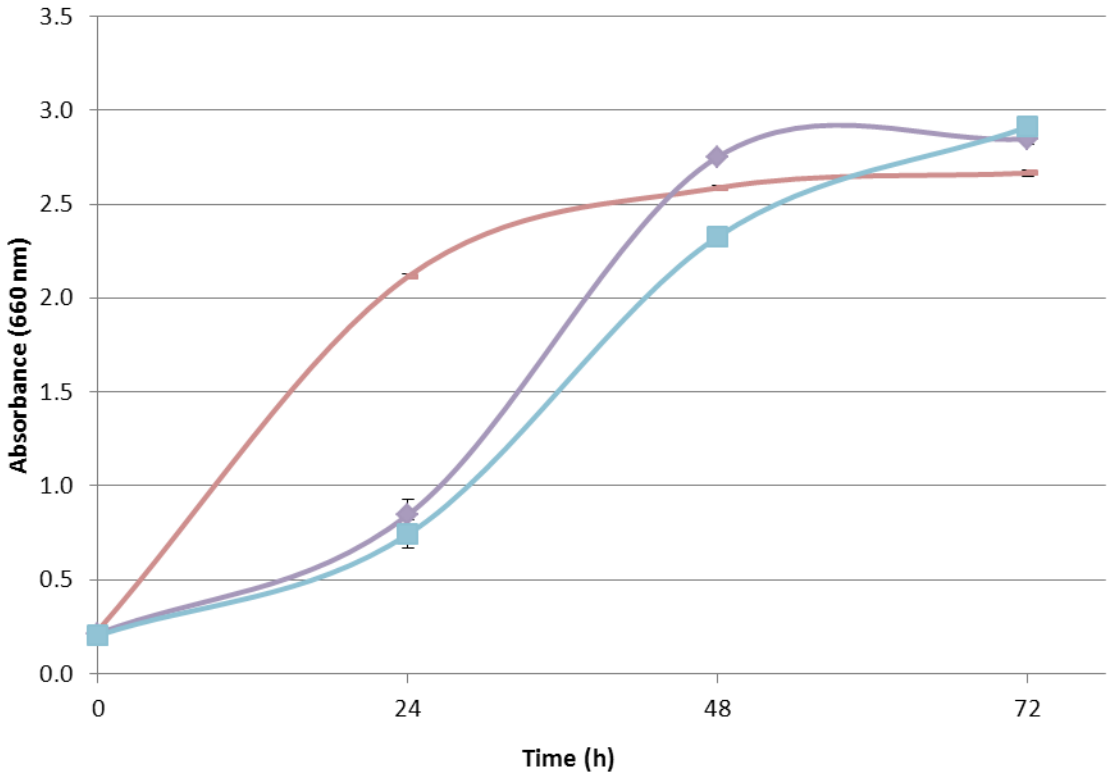


Figure 3.1: Growth profiles of *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 OC under anaerobic conditions and continuous illumination at 2000 lux.

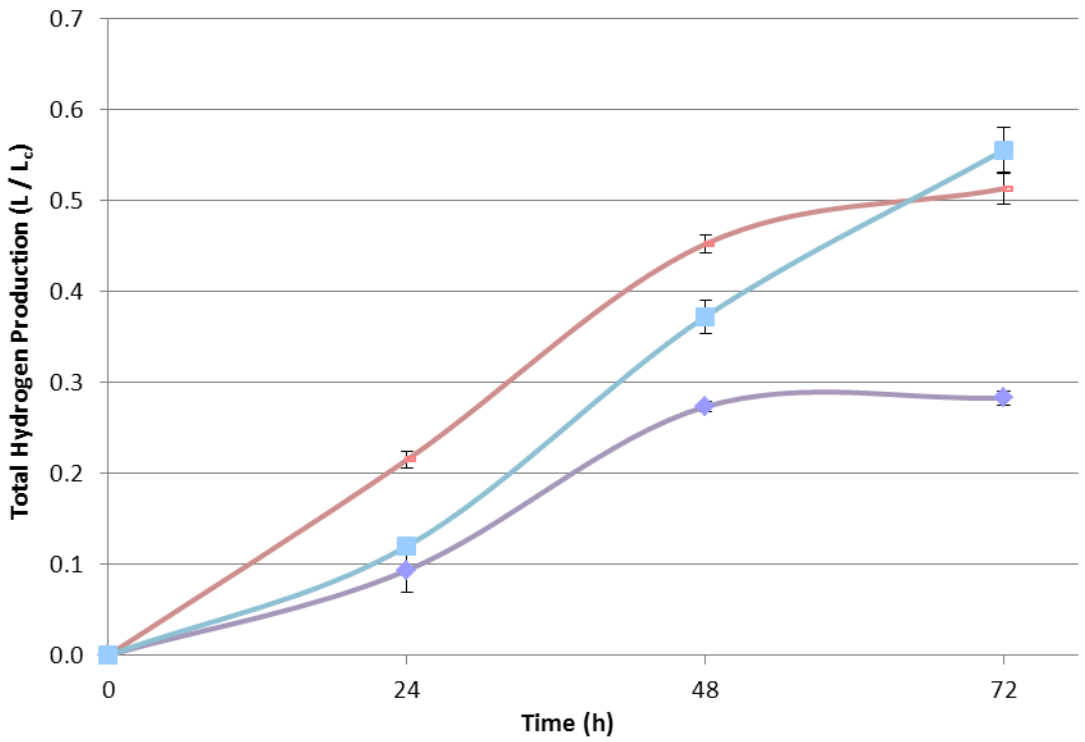


Figure 3.2: Total hydrogen production of *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

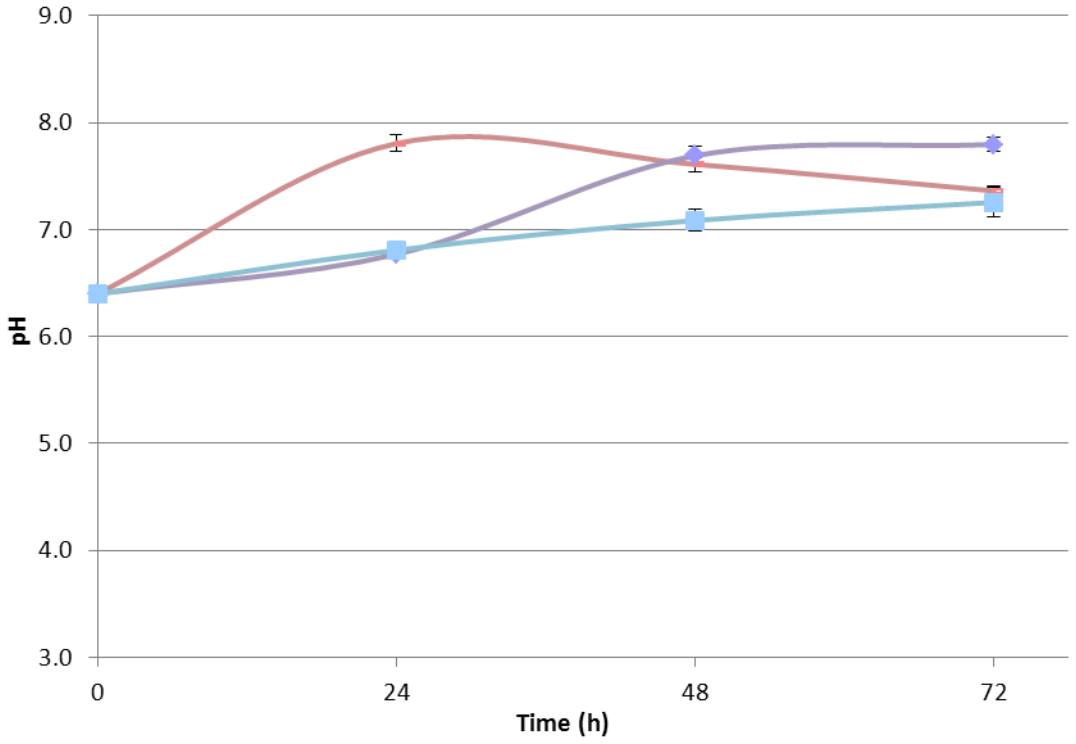


Figure 3.3: pH values of *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Table 3.1: Maximum Biomass, Total Produced Hydrogen, Hydrogen Productivity and Specific Growth Rate values of single cultures of PNS bacteria cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Microorganisms	Maximum Biomass (gdcw / L_c)	Total Produced H_2(L / L_c)	Hydrogen Productivity (mmol / L.h)	Specific Growth Rate (h^{-1})
R. capsulatus hup-	1.24±0.01	0.51±0.19	0.13±0.00	0.092±0.001
R. palustris	0.74±0.01	0.28±0.01	0.10±0.01	0.050±0.004
R. sphaeroides	1.68±0.02	0.56±0.03	0.14±0.01	0.048±0.004

3.1.2 Growth, Hydrogen Production on Hydrogen Production Medium

Experiments on hydrogen production medium were conducted in order to compare hydrogen production performances of PNS bacteria. This medium has contained 40 mM acetate and 2 mM glutamate. Hence the C/N ratio of this medium was 10 times higher than the C/N ratio of the 20 mM acetate/10 mM glutamate growth medium. Lower glutamate concentration was supplied in order to maintain sufficient growth, but also to prevent inhibition of hydrogen production due to nitrogen afterwards. Also higher acetate concentration was supplied in order to maintain high amount of carbon source, for conversion of more substrate to hydrogen. *R. capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) grew on hydrogen production medium without a lag phase (Figure 3.4), while *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) exhibited 24 hour lag phase. This lag phase has been found to be reflected on the pH (Fgure 3.6) and total hydrogen production values of *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) as 48 hour lags (Figure 3.5). Despite the 48 hours lag, these cultures were found to complete hydrogen production at the same time with other

strains; hence their durations of hydrogen production were shorted than other strains. Additionally pH values were found to be within potassium phosphate buffer capacity. The maximum growth rates calculated were: 0.006±0.000, 0.007±0.000, 0.005±0.001 and 0.016±0.003 h^{-1} for *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) respectively (Table 3.2). These values have been found much lower than the growth rates obtained by experimentations performed on growth medium. Maximum biomass concentrations were similarly found slightly lower than the maximum biomass concentrations obtained from experimentations on growth medium with 1.10±0.03, 1.12±0.05, 0.64±0.02 and 1.57±0.05 gdcw / L_c for *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) respectively. Total hydrogen production data were: 1.63±0.10, 0.92±0.09, 1.73±0.09 and 1.48±0.04 L / L_c for *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) respectively. Also the hydrogen productivities were found as 0.52±0.07, 0.23±0.04, 0.54±0.01 and 0.51±0.03 mmol / L.h for *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) respectively. Hydrogen yields were found between 1.93±0.10 and 1.03±0.10 mol H_2 / mol acetate, substrate conversion efficiencies were found between 48.30±2.52 and 25.78±2.51 % and light conversion efficiencies were found between 0.48±0.01 and 0.20±0.03 %. Total hydrogen production, hydrogen productivity, hydrogen yield, substrate conversion efficiency and light conversion efficiency data have correlated with each other. Total hydrogen production and hydrogen productivity results were also significantly higher than the results obtained from experimentations on growth medium. All single cultures of PNS bacteria have depleted all of their substrates (acetate) completely within 144 hour. Rates of substrate consumption were not significantly different; however *R. capsulatus* hup- (YO3) and *R.*

capsulatus (DSM 1710) seem to utilize acetate at a faster rate during initial 24 hour period, which is accordance with the growth profiles (Figure 3.7). These results together show that, PNS bacteria are biased towards biomass accumulation on growth medium, and towards hydrogen production on hydrogen production medium. Also by comparison of total hydrogen production data for growth and hydrogen production media, one may imply that, *R. palustris* (DSM 127) exhibits better total hydrogen production than others on higher acetate concentrations, and *R. sphaeroides* O.U.001 (DSM 5864) exhibits better total hydrogen production than others on lower acetate concentrations. When different PNS bacteria strains on hydrogen production medium are compared, the strains which exhibited the highest total hydrogen productions, hydrogen yields, substrate conversion efficiencies, hydrogen productivities and light conversion efficiencies were found to exhibit the lowest maximum biomasses and specific growth rates. Hence metabolisms of PNS bacteria cultivated on hydrogen production medium were either found to be biased towards biomass growth or hydrogen production too. One exception was *R. sphaeroides* O.U.001 (DSM 5864), which have exhibited significantly higher biomass and specific growth rate values than single cultures of other PNS bacteria, however also significantly higher values than *R. capsulatus* (DSM 1710) for all other data mentioned. Therefore *R. sphaeroides* O.U.001 (DSM 5864) was one of the most active strains; however have biased biomass formation rather than hydrogen production.

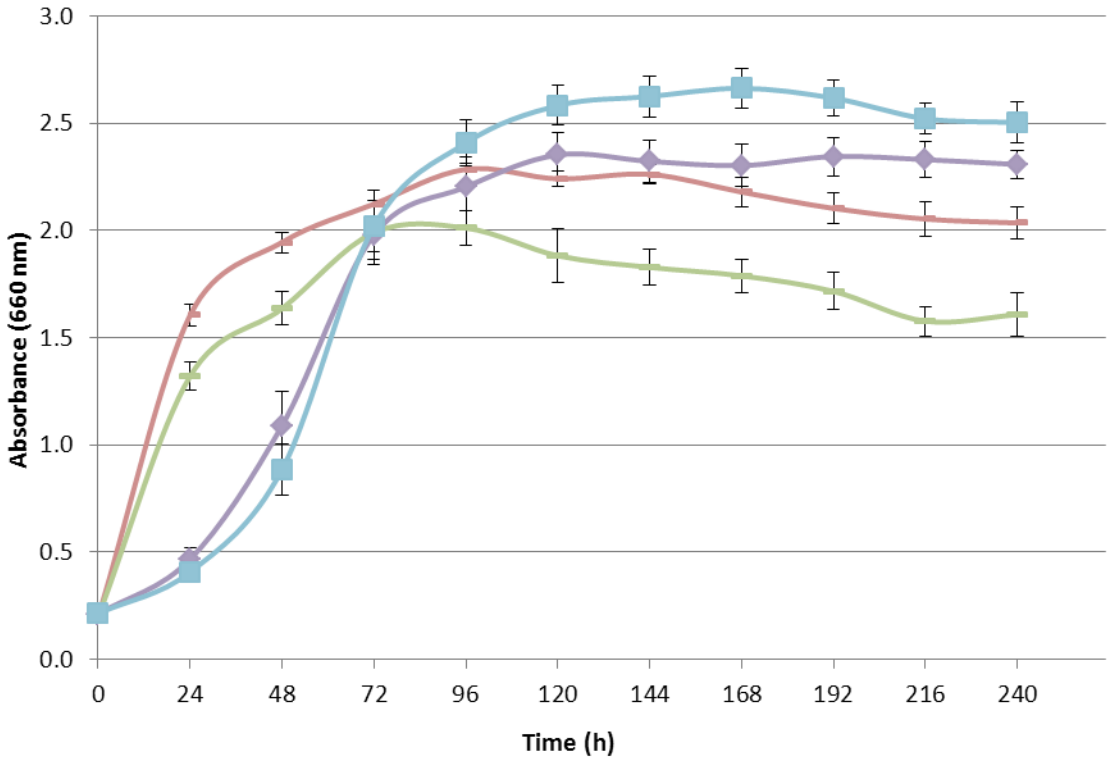


Figure 3.4: Growth profiles of *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

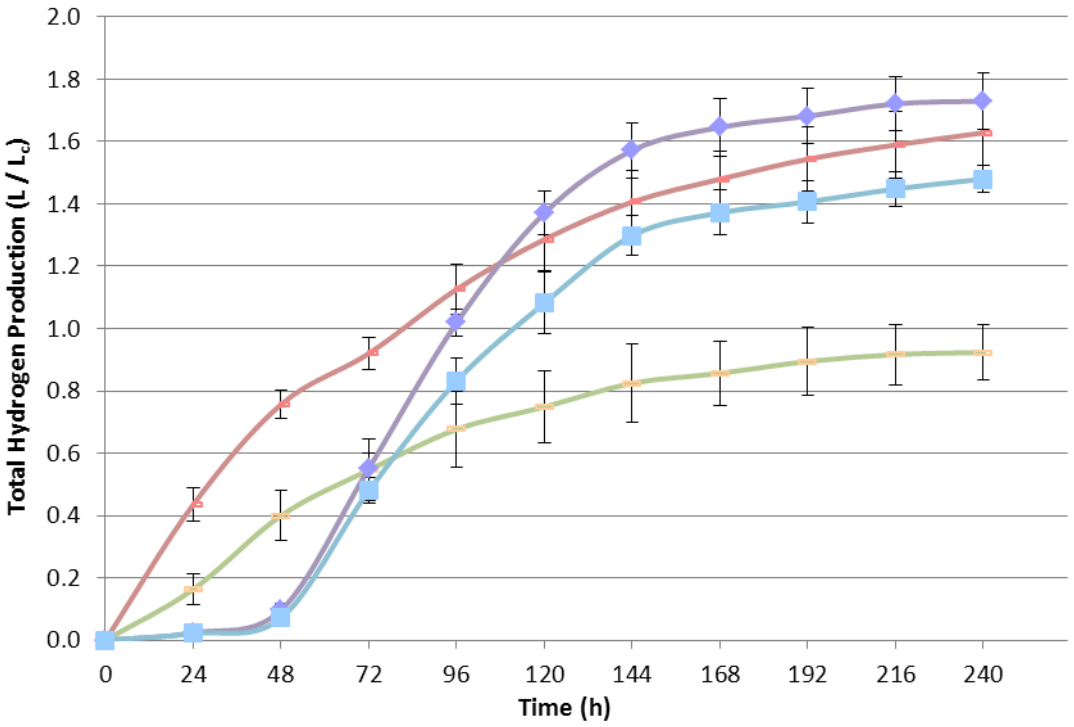


Figure 3.5: Total hydrogen production of *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

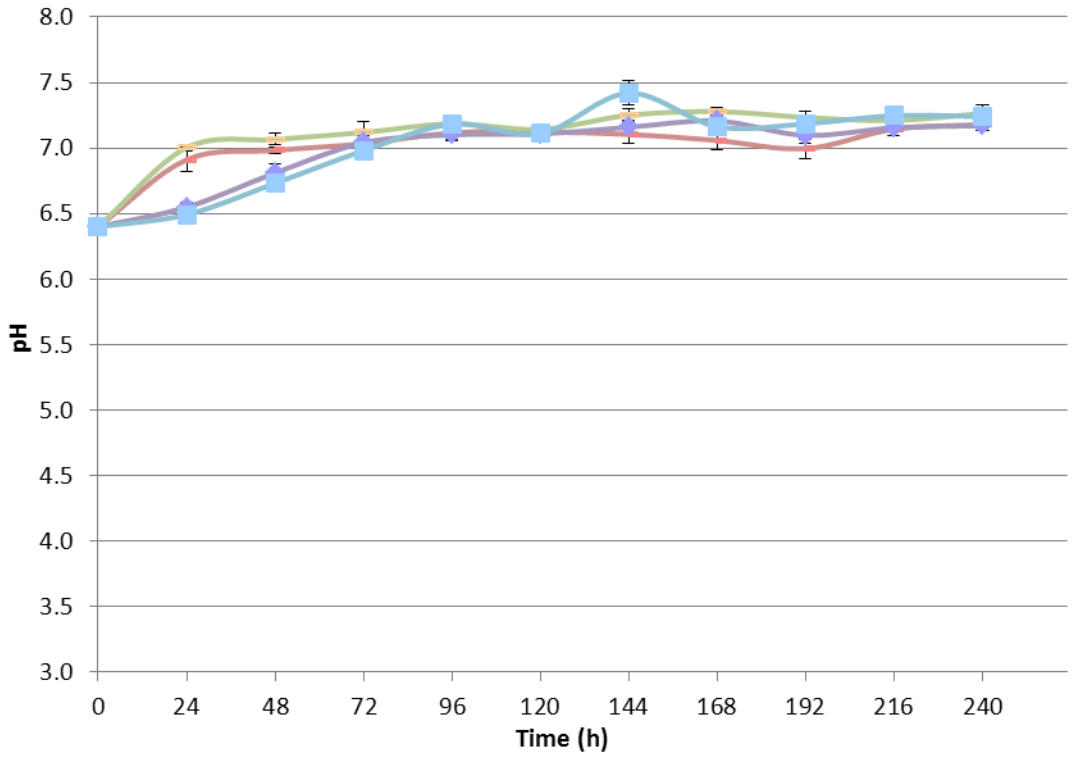


Figure 3.6: pH values of *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

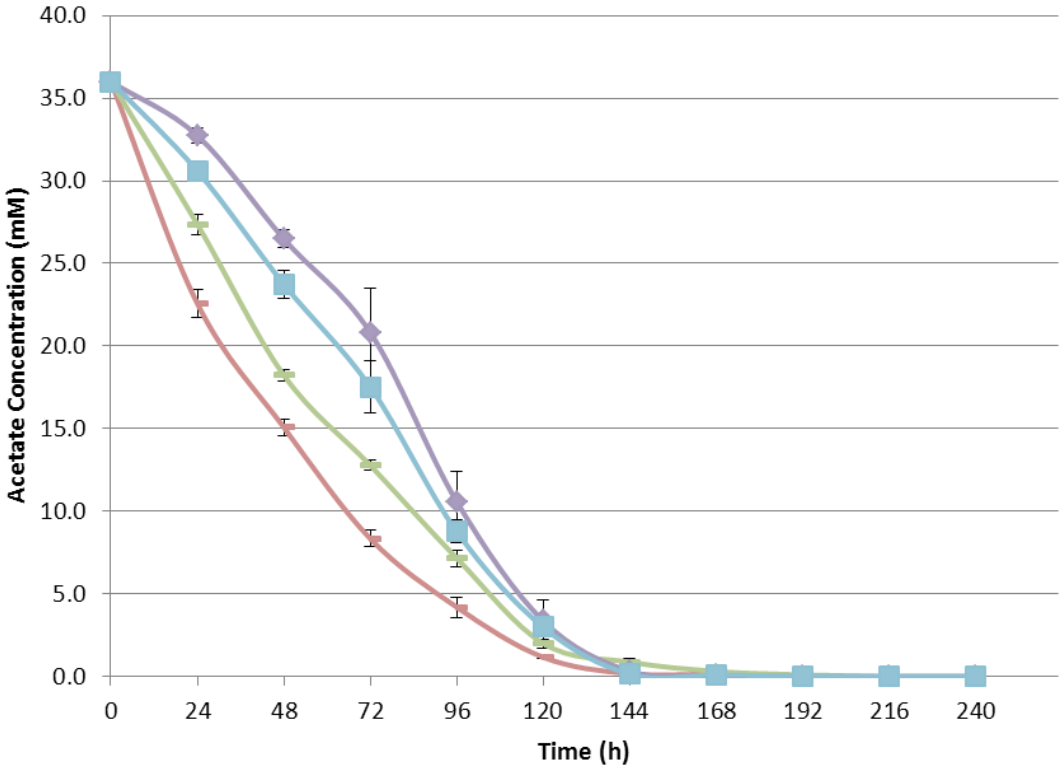


Figure 3.7: Acetate Consumption of *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Table 3.2: Maximum Biomass, Total Produced Hydrogen, Hydrogen Yield, Substrate Conversion Efficiency, Duration of Hydrogen Production, Hydrogen Productivity, Light Conversion Efficiency and Specific Growth Rate values of single cultures of PNS bacteria cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Microorganisms	Maximum Biomass (gdcw / L_c)	Total Produced H_2(L / L_c)	Hydrogen Yield (mol H_2 / mol Acetate)	Substrate Conversion Efficiency (%)	Duration of Hydrogen Production (h)	Hydrogen Productivity (mmol / L.h)	Light Conversion Efficiency (%)	Specific Growth Rate (h^{-1})
R. capsulatus hup-	1.10±0.03	1.63±0.10	1.82±0.12	45.47±2.91	144	0.52±0.07	0.46±0.06	0.006±0.000
R. capsulatus	1.12±0.05	0.92±0.09	1.03±0.10	25.78±2.51	144	0.23±0.04	0.20±0.03	0.007±0.000
R. palustris	0.64±0.02	1.73±0.09	1.93±0.10	48.30±2.52	96	0.54±0.01	0.48±0.01	0.005±0.001
R. sphaeroides	1.57±0.05	1.48±0.04	1.65±0.05	41.32±1.19	96	0.51±0.03	0.46±0.03	0.016±0.003

3.2 Co-cultivation of Two PNS Bacteria Strains on Hydrogen Production Medium

Different combination of PNS bacteria strains were co-cultured on hydrogen production medium containing 40 mM acetate and 2 mM glutamate as carbon and nitrogen sources, respectively. Combinations of bacteria are given in Table 3.3.

Table 3.3: Co-cultures of two different strains of PNS bacteria cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Co-cultures of two different strains of PNS bacteria	*R. capsulatus* hup- + *R. palustris*
	R. capsulatus hup- + *R. sphaeroides*
	R. capsulatus + *R. palustris*
	R. capsulatus + *R. sphaeroides*
	R. palustris + *R. sphaeroides*

Experiments of co-cultures of two different PNS bacteria have shown that lag phase was not observed for growth profiles in any co-culture combination (Figure 3.8), including *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), which single cultures of the strains constituting it have exhibited 24 hour lag phases. This might be due to increased startup biomass concentrations when compared with the single cultures. Also acetate utilization data of all groups were found highly similar (Figure 3.11). All groups have depleted their substrates completely within 144 hours. However, a 24 hour lag phase was observed for *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) when total hydrogen production results have been investigated (Figure 3.9). Although the initial biomass concentration seems adequate for overcoming the lag phase for growth, nitrogen source seems to be not depleted during first 24 hour, and did not allow hydrogen production. Despite this 24 hour lag, *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) has completed hydrogen production 24 hour earlier than other co-cultures, hence the duration of hydrogen production was observed 48 hour shorter. pH values of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) also have correlated with total hydrogen production results, *R. palustris* (DSM 127) + *R.*

sphaeroides O.U.001 (DSM 5864) exhibiting slower pH rise than other co-cultures of two different strains of PNS bacteria, which include *R. capsulatus* hup- (YO3) or *R. capsulatus* (DSM 1710). Additionally pH values were found to be within the buffer capacity of the potassium phosphate buffer (Figure 3.10). Determination of specific growth rates has shown that maximum growth rates have varied between 0.004±0.000 and 0.011±0.001 h^{-1} (Table 3.4). The lowest maximum growth rate was observed for *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), which has exhibited the highest total hydrogen production among co-cultures of two different strains of PNS bacteria with 1.77±0.08 L / L_c, and the highest maximum growth rates were observed for the co-cultures including *R. capsulatus* (DSM 1710), which have exhibited the lowest total hydrogen production with 1.50±0.12 and 1.55±0.01 L / L_c. Maximum biomasses have varied between 1.12±0.05 and 1.70±0.09 gdcw / L_c, among which, the co-cultures including *R. palustris* (DSM 127) have resulted in lower maximum biomass growths than the others. When total hydrogen production results obtained are compared with the single cultures of PNS bacteria, an overall significant improvement may not be observed. This is due to less amount of medium and higher amount of inoculum added to photobioreactors for co-cultures. Hence hydrogen yields and substrate conversion efficiencies are key variables for comparison of hydrogen production performances of single and co-cultures of PNS bacteria. Hydrogen yields varying between 2.23±0.10 and 1.88±0.15 mol H_2 / mol acetate and substrate conversion efficiencies varying between 55.64±2.57 and 47.11±3.82 %, which have also correlated with total hydrogen production values, have exhibited increase in hydrogen production performances over single cultures. Maximum hydrogen production rates on the other hand, have varied between 0.94±0.06 and 0.45±0.03 mmol / L.h, and the results for hydrogen productivity have correlated light conversion efficiencies varying between 0.84±0.05 and 0.40±0.02 %. Maximum hydrogen production and light conversion efficiency values also have

mostly correlated with total produced hydrogen results of the co-cultures of two different PNS bacteria. One exception was *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) co-culture which exhibited lower hydrogen productivity than *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127). Co-cultures involving two different strains of PNS bacteria have exhibited higher hydrogen productivities than single cultures, except the ones including *R. capsulatus* (DSM 1710), which fall behind the hydrogen productivities of single cultures of *R. palustris* (DSM 127) or *R. sphaeroides* O.U.001 (DSM 5864). All co-cultures of two different PNS bacteria have utilized their substrates completely with very similar rates. In conclusion, *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) has exhibited the highest total hydrogen production, hydrogen yield, substrate conversion efficiency, hydrogen productivity and light conversion efficiency, whereas the slowest growth rate and second lowest maximum biomass growth, although its maximum biomass growth was not significantly different from the other co-cultures including *R. palustris* (DSM 127). In general, co-cultures of two different strains of PNS bacteria have biased towards biomass growth or hydrogen production, similarly to the single culture results except the co-cultures of *R. sphaeroides* O.U.001 (DSM 5864) with *R. capsulatus* hup- (YO3) or *R. capsulatus* (DSM 1710) which have exhibited higher hydrogen production and biomass growth than the co-cultures *R. palustris* (DSM 127) with *R. capsulatus* hup- (YO3) or *R. capsulatus* (DSM 1710), and comparable specific growth rates. Hence the co-cultures of *R. sphaeroides* O.U.001 (DSM 5864) have exhibited higher activity in overall than *R. palustris* (DSM 127). However, as the hydrogen productivity and light conversion efficiency data reveals, the maximal activity of *R. palustris* (DSM 127) co-cultured with *R. capsulatus* hup- (YO3) was found to be higher than the maximal activity of *R. sphaeroides* O.U.001 (DSM 5864) co-cultured with *R. capsulatus* hup- (YO3).

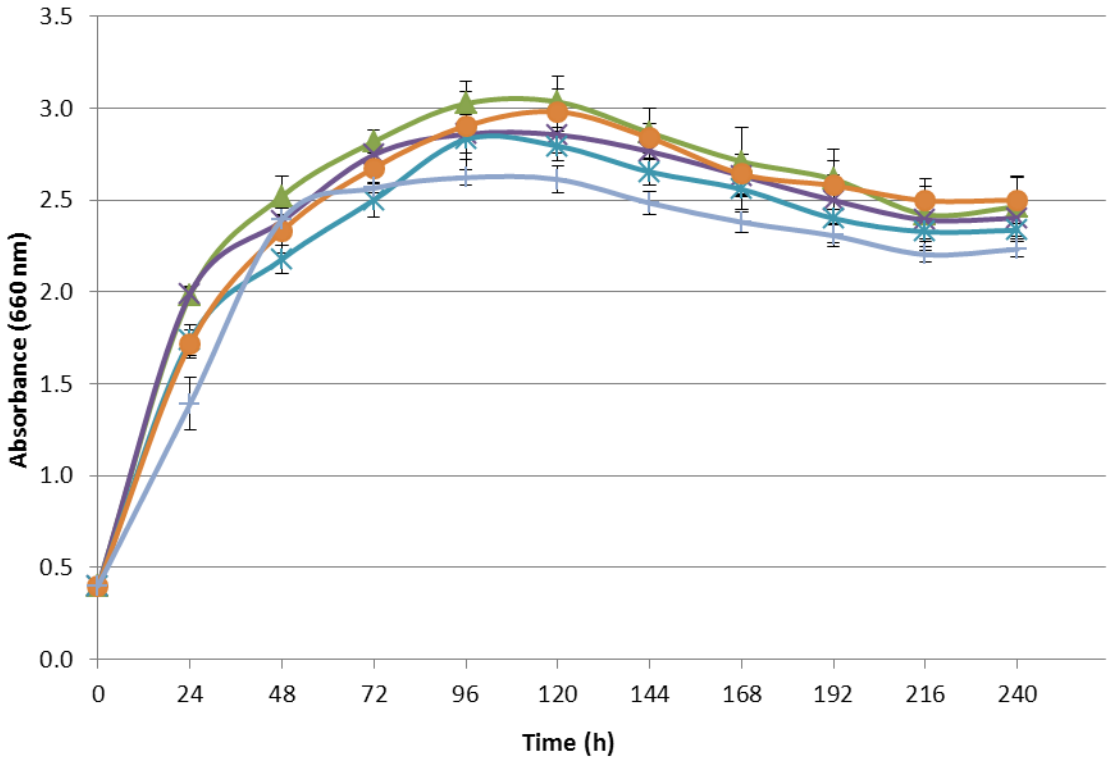


Figure 3.8: Growth profiles of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127), *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

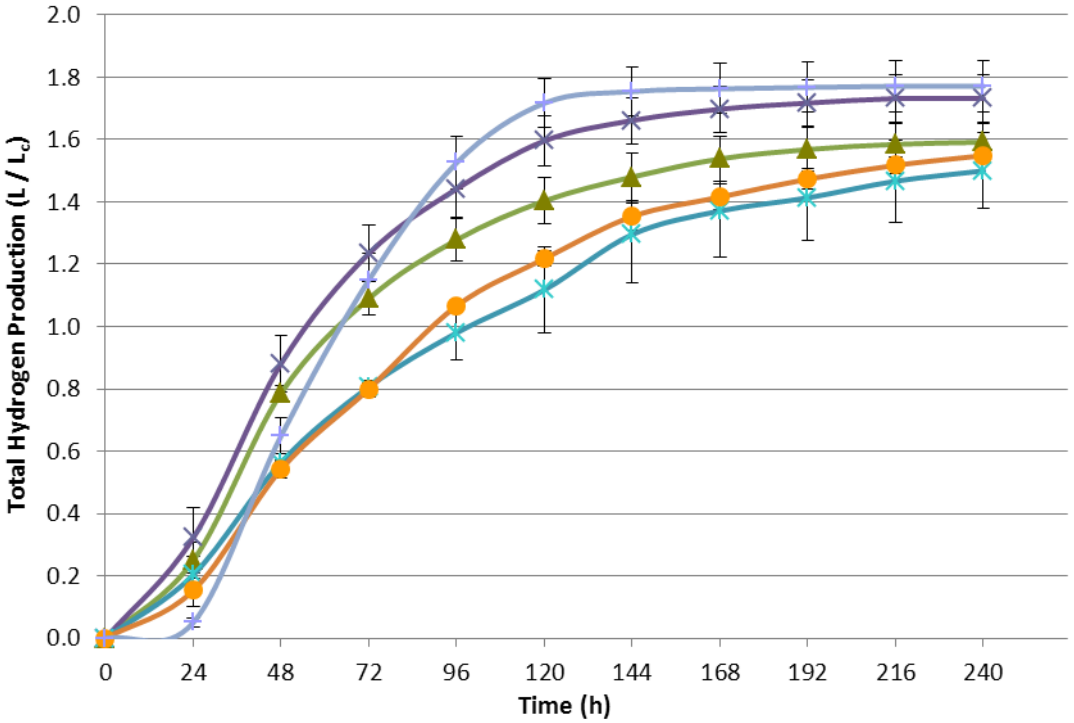


Figure 3.9: Total hydrogen production of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127), *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

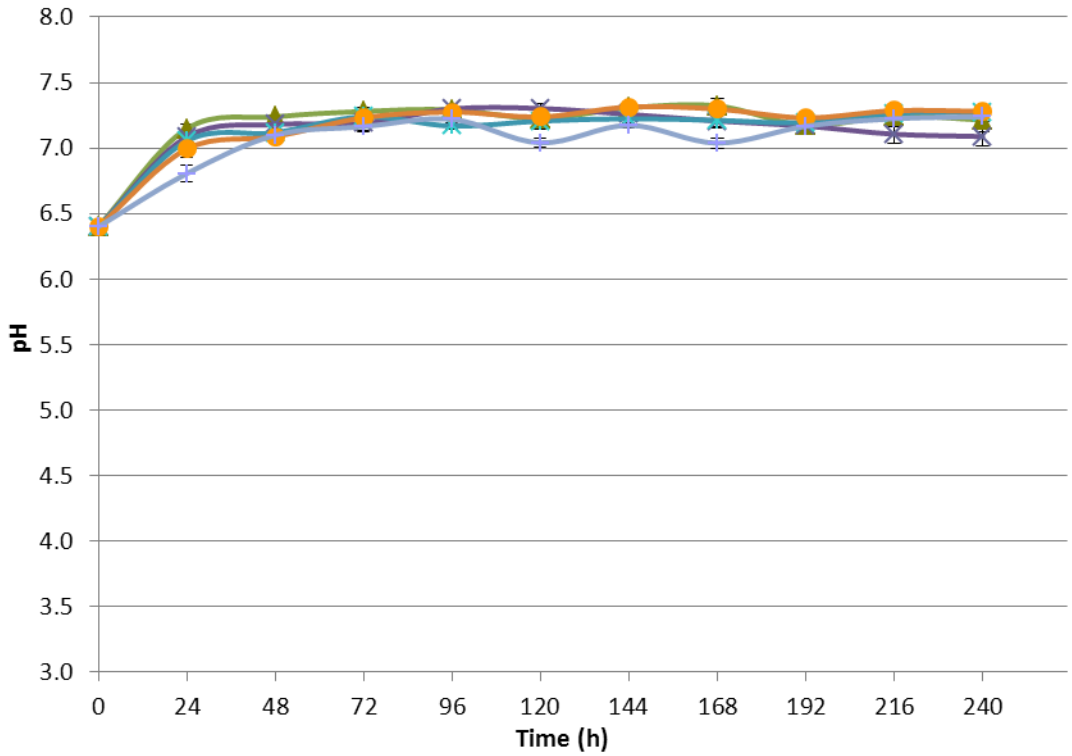


Figure 3.10: pH values of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127), *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

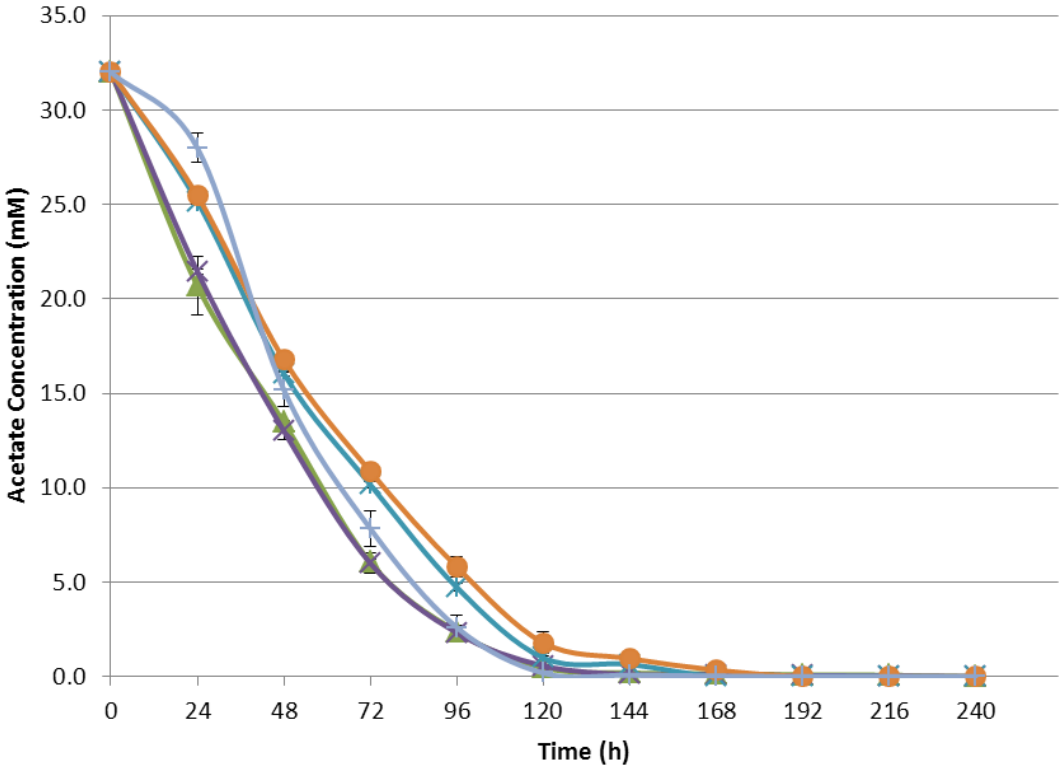


Figure 3.11: Acetate Consumption of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127), *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Table 3.4: Maximum Biomass, Total Produced Hydrogen, Hydrogen Yield, Substrate Conversion Efficiency, Duration of Hydrogen Production, Hydrogen Productivity, Light Conversion Efficiency and Specific Growth Rate values of two different strains of PNS bacteria cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Microorganisms	Maximum Biomass (gdcw / L_c)	Total Produced H_2(L / L_c)	Hydrogen Yield (mol H_2 / mol Acetate)	Substrate Conversion Efficiency (%)	Duration of Hydrogen Production (h)	Hydrogen Productivity (mmol / L.h)	Light Conversion Efficiency (%)	Specific Growth Rate (h^{-1})
R. capsulatus hup- + *R. palustris*	1.12±0.05	1.59±0.06	2.00±0.07	49.99±1.83	144	0.75±0.05	0.67±0.04	0.009±0.000
R. capsulatus hup- + *R. sphaeroides*	1.52±0.05	1.73±0.08	2.18±0.09	54.39±2.36	144	0.66±0.03	0.58±0.03	0.009±0.000
R. capsulatus + *R. palustris*	1.14±0.06	1.50±0.12	1.88±0.15	47.11±3.82	144	0.45±0.02	0.40±0.02	0.011±0.001
R. capsulatus + *R. sphaeroides*	1.70±0.09	1.55±0.01	1.95±0.02	48.65±0.39	144	0.45±0.03	0.40±0.02	0.011±0.001
R. palustris + *R. sphaeroides*	1.13±0.02	1.77±0.08	2.23±0.10	55.64±2.57	96	0.94±0.06	0.84±0.05	0.004±0.000

3.3 Co-cultivation of Three Different Strains of PNS Bacteria Hydrogen Production Medium

Three different strains of PNS bacteria were co-cultivated on hydrogen production medium (Table 3.5.). Two different sets of co-cultures were prepared, one containing *R. capsulatus* (DSM 1710) and the other containing *R. capsulatus* hup- (YO3). The other strains (*R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864)) were the same in both. Hence the difference in hydrogen production will reflect the effect of uptake hydrogenase deletion.

Table 3.5: Co-cultures of three different strains of PNS bacteria cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Co-cultures of three different strains of PNS bacteria	*R. capsulatus* hup- + *R. palustris* + *R. sphaeroides*
	R. capsulatus + *R. palustris* + *R. sphaeroides*

Co-cultures of three different strains of PNS bacteria have exhibited very comparable results. Both co-cultures have exhibited similar growth (Figure 3.12), hydrogen production (Figure 3.13), pH (Figure 3.14) and acetate utilization results (Figure 3.15), where lag phases have not been observed. Specific growth rates were calculated as 0.011±0.001 h^{-1}, hydrogen productivities were calculated as 0.90±0.02 and 0.82±0.02 mmol / L.h and maximum biomasses were calculated between 1.43±0.05 and 1.49±0.03 gdcw / L_c (Table 3.6). Specific growth rates were higher than the single cultures except *R. sphaeroides* O.U.001 (DSM 5864), and were comparable with the highest specific growth rates observed for co-cultures of two different strains of PNS bacteria. Hydrogen productivities were calculated as 0.90±0.02 and 0.82±0.02, and were also found higher than all other single cultures and co-cultures, except *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864). Maximum biomasses were found between the highest and the lowest biomasses observed for single cultures and co-cultures of two different strains of PNS bacteria, however higher than the average of single cultures and co-cultures of two different strains of PNS bacteria. Total hydrogen production values were 1.82±0.04 L / L_c for *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) and 1.74±0.03 L / L_c for *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), which were higher than

all other single and co-cultures except *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), which was slightly higher but comparable to *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864). Additionally single culture *R. palustris* (DSM 127) and co-culture *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) have exhibited very comparable total hydrogen production results with *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864). However hydrogen yields of 2.61±0.06 and 2.50±0.05 mol H_2 / mol acetate and substrate conversion efficiencies of 65.28±1.42 and 62.52±1.14 % were found to be significantly higher than other single and co-cultures. The reason of this was the lower initial substrate concentrations of the triple cultures of the PNS bacteria, due to less amount of medium and higher amount of bacterial inoculum addition to the photobioreactors, when compared with the single and co-cultures of two different PNS bacteria. Hydrogen productivities were found as 0.90±0.02 and 0.82±0.02 mmol / L.h and light conversion efficiencies were found as 0.80±0.02 and 0.73±0.02 %, which were also higher than all other groups, except the *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) co-culture. Additionally pH values were found within the buffer capacity of the potassium phosphate buffer, and the substrates were completely utilized within 120 hours. Among the co-cultures of three different strains of PNS bacteria, *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) has exhibited higher hydrogen production, and *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) has exhibited higher biomass growth.

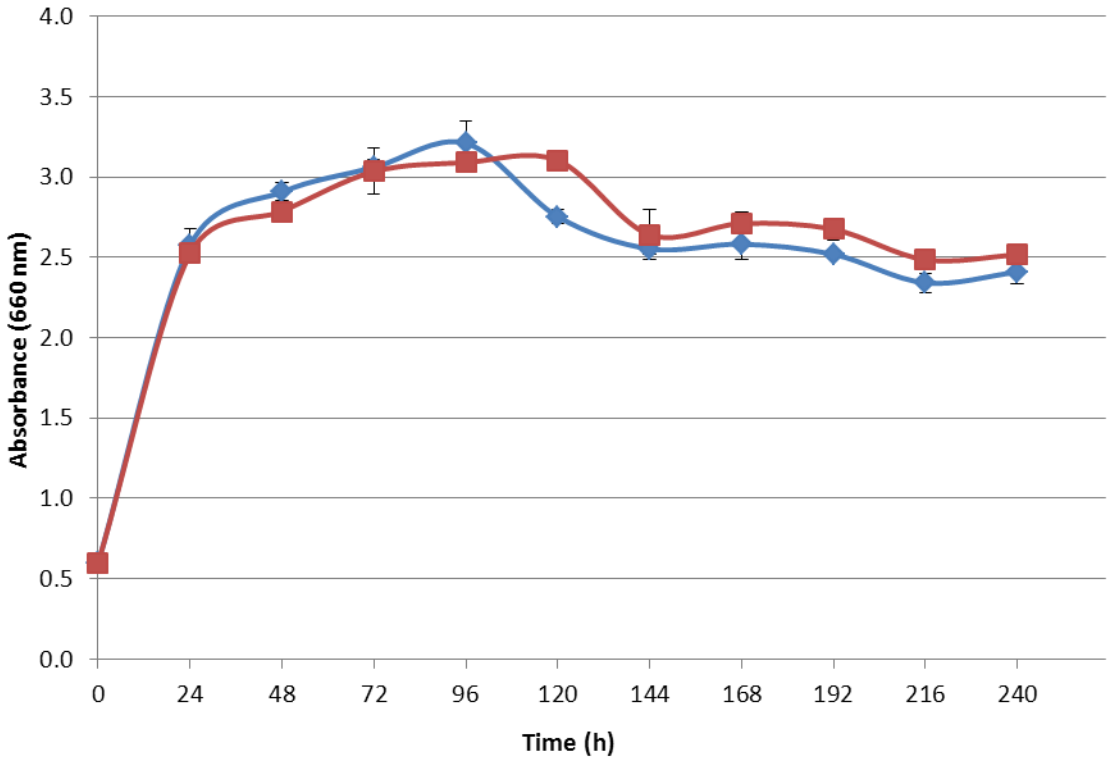


Figure 3.12: Growth profiles of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

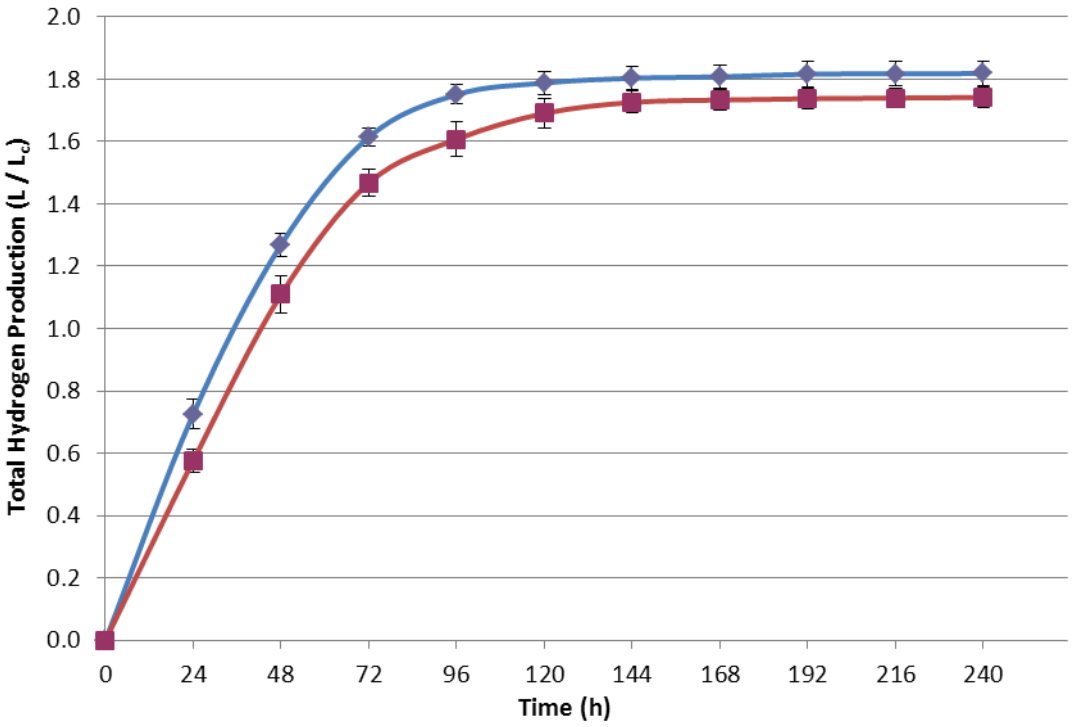


Figure 3.13: Total hydrogen production of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

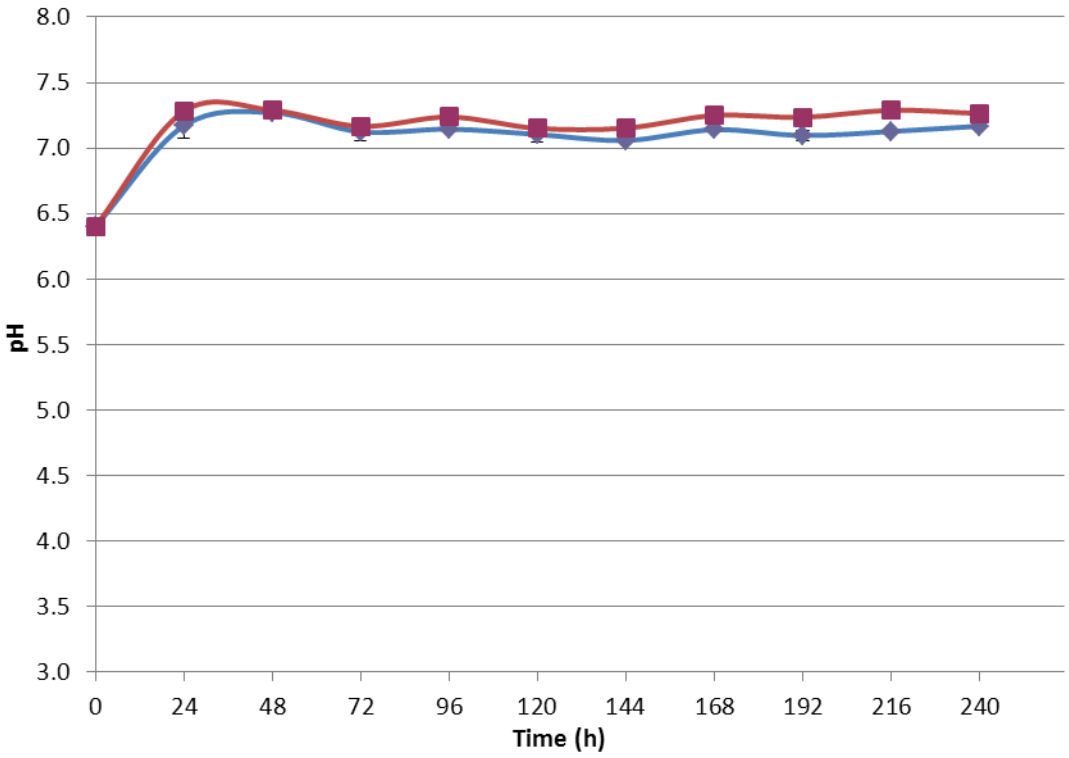


Figure 3.14: pH values of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

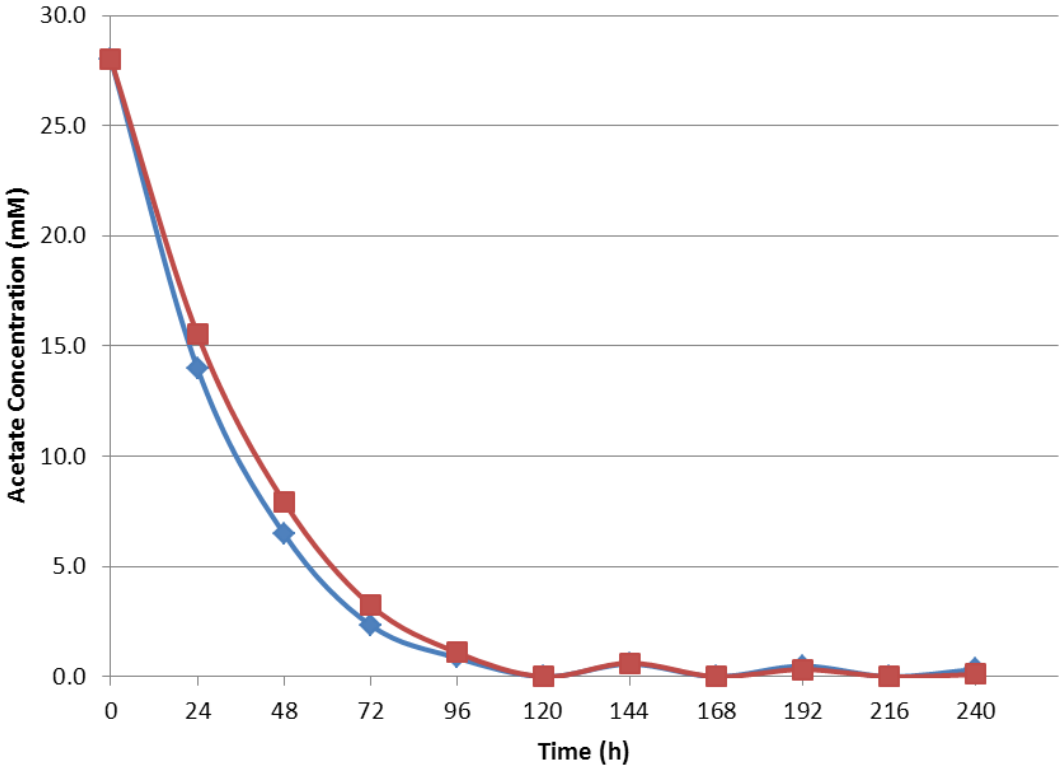


Figure 3.15: Acetate Consumption of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Table 3.6: Maximum Biomass, Total Produced Hydrogen, Hydrogen Yield, Substrate Conversion Efficiency, Duration of Hydrogen Production, Hydrogen Productivity, Light Conversion Efficiency and Specific Growth Rate values of co-cultures of three different strains of PNS bacteria cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Microorganisms	Maximum Biomass (gdcw / L_c)	Total Produced H_2(L / L_c)	Hydrogen Yield (mol H_2 / mol Acetate)	Substrate Conversion Efficiency (%)	Duration of Hydrogen Production (h)	Hydrogen Productivity (mmol / L.h)	Light Conversion Efficiency (%)	Specific Growth Rate (h^{-1})
R. capsulatus hup- + *R. palustris* + *R. sphaeroides*	1.43±0.05	1.82±0.04	2.61±0.06	65.28±1.42	96	0.90±0.02	0.80±0.02	0.011±0.001
R. capsulatus + *R. palustris* + *R. sphaeroides*	1.49±0.03	1.74±0.03	2.50±0.05	62.52±1.14	96	0.82±0.02	0.73±0.02	0.011±0.000

3.4 Comparison of the Hydrogen Production Performances for Single Cultures, Co-Cultures of Two and Three Different Strains of PNS Bacteria on Hydrogen Production Medium

A summary of the comparison of hydrogen production capacities of single cultures, co-cultures of two and three different strains of PNS bacteria is given in Table 3.3.

For experiments carried out with the addition of the same amounts of substrates, substrate conversion efficiencies would be proportional to total hydrogen productions. Also in cases which the added substrate was utilized completely, such as the experiments performed in this study, hydrogen yield would be proportional to total hydrogen production too.

However in this study, although hydrogen yields and substrate conversion efficiencies were parallel, they were not parallel to total hydrogen production. This was due to that, different amounts of bacteria were inoculated to different amounts of media, depending on the number different bacteria to be cultivated or co-cultivated together. Comparison of the performances of single cultures and co-cultures of the bacteria in this study; hence was possible via comparison of their hydrogen yields.

Table 3.7: Maximum Biomass, Total Produced Hydrogen, Hydrogen Yield, Substrate Conversion Efficiency, Duration of Hydrogen Production, Hydrogen Productivity, Light Conversion Efficiency and Specific Growth Rate values of PNS bacteria cultured on hydrogen production medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Type of Culture	Microorganisms	Maximum Biomass (gdcw / L_c)	Total Produced H_2(L / L_c)	Hydrogen Yield (mol H_2 / mol Acetate)	Substrate Conversion Efficiency (%)	Duration of Hydrogen Production (h)	Hydrogen Productivity (mmol / L.h)	Light Conversion Efficiency (%)	Specific Growth Rate (h^{-1})
Single Cultures of PNS Bacteria	*R. capsulatus* hup-	1.10±0.03	1.63±0.10	1.82±0.12	45.47±2.91	144	0.52±0.07	0.46±0.06	0.006±0.000
	R. capsulatus	1.12±0.05	0.92±0.09	1.03±0.10	25.78±2.51	144	0.23±0.04	0.20±0.03	0.007±0.000
	R. palustris	0.64±0.02	1.73±0.09	1.93±0.10	48.30±2.52	96	0.54±0.01	0.48±0.01	0.005±0.001
	R. sphaeroides	1.57±0.05	1.48±0.04	1.65±0.05	41.32±1.19	96	0.51±0.03	0.46±0.03	0.016±0.003
Co-Cultures of Two Different PNS Bacteria	*R. capsulatus* hup- + *R. palustris*	1.12±0.05	1.59±0.06	2.00±0.07	49.99±1.83	144	0.75±0.05	0.67±0.04	0.009±0.000
	R. capsulatus hup- + *R. sphaeroides*	1.52±0.05	1.73±0.08	2.18±0.09	54.39±2.36	144	0.66±0.03	0.58±0.03	0.009±0.000
	R. capsulatus + *R. palustris*	1.14±0.06	1.50±0.12	1.88±0.15	47.11±3.82	144	0.45±0.02	0.40±0.02	0.011±0.001
	R. capsulatus + *R. sphaeroides*	1.70±0.09	1.55±0.01	1.95±0.02	48.65±0.39	144	0.45±0.03	0.40±0.02	0.011±0.001
	R. palustris + *R. sphaeroides*	1.13±0.02	1.77±0.08	2.23±0.10	55.64±2.57	96	0.94±0.06	0.84±0.05	0.004±0.000
Co-Cultures of Three Different PNS Bacteria	*R. capsulatus* hup- + *R. palustris* + *R. sphaeroides*	1.43±0.05	1.82±0.04	2.61±0.06	65.28±1.42	96	0.90±0.02	0.80±0.02	0.011±0.001
	R. capsulatus + *R. palustris* + *R. sphaeroides*	1.49±0.03	1.74±0.03	2.50±0.05	62.52±1.14	96	0.82±0.02	0.73±0.02	0.011±0.000

Also in this study, light conversion efficiency data was parallel with hydrogen productivity data, since the density of the hydrogen gas, illumination, and the illuminated area were constant for experimented single cultures and co-cultures, and the duration of the hydrogen production were the same, and the volume and the milimolar amount of the produced gas were proportional for each single culture and co-culture group.

As seen in the Table (3.6), *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) was the co-culture of three different PNS bacteria, which has exhibited the highest total hydrogen production with 1.82±0.04 L / L_c, the highest hydrogen yield with 2.61±0.06 mol H_2 / mol acetate, and the highest substrate conversion efficiency with 65.28±1.42 %, among all experimented groups. Hence it was the experimented group which was favored hydrogen production more than any other single culture or co-cultures of PNS bacteria. The total hydrogen production of it was found to exhibit 1.12 fold improvements over co-cultures of two different PNS bacteria and 1.26 fold improvements over single cultures. Whereas hydrogen yield was found to be improved 1.27 fold over co-cultures of two different strains of PNS bacteria, and 1.62 fold over single cultures of PNS bacteria. Additionally substrate conversion efficiency was found to be improved 1.28 fold over single cultures and 1.62 fold over co-cultures of two different PNS bacteria.

R. palustris (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) on the other hand, has exhibited the highest hydrogen productivity with 0.94±0.06 mmol / L.h and the highest light conversion efficiency with 0.84±0.05 %, and the lowest specific growth rate with 0.004±0.000 h^{-1} among all experimented groups. Hence it has exhibited highest hydrogen production maxima and the lowest growth maxima. *R. palustris* (DSM 127) +

R. sphaeroides O.U.001 (DSM 5864) has also exhibited the highest total hydrogen production with 1.77±0.08 L / L_c, also the highest hydrogen yield and substrate conversion efficiency values among co-cultures of two different PNS bacteria. Hydrogen productivity for *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) was observed to be improved 2.09 fold over single cultures, whereas light conversion efficiency was found to be improved 2.10 fold over single cultures. Additionally total hydrogen production was improved 1.23 fold over single cultures and specific growth rate was recessed 2.13 old over single cultures.

The lowest biomass growth was exhibited by *R. sphaeroides* O.U.001 (DSM 5864) with 0.64±0.02 gdcw / L_c, which also has exhibited the highest total hydrogen production, hydrogen yield, substrate conversion efficiency, hydrogen productivity and light conversion efficiency values among single cultures of PNS bacteria. Hence *R. sphaeroides* O.U.001 (DSM 5864) has biased hydrogen production instead of biomass growth, when compared with single cultures of other PNS bacteria.

The best co-culture partner might be suggested to be *R. sphaeroides* O.U.001 (DSM 5864), although it ranks the third in single culture experiments. All co-cultures which *R. sphaeroides* O.U.001 (DSM 5864) was one of the partners have led to higher yields when compared with the co-cultures of other PNS bacteria with the other partner. Also top four co-cultures for hydrogen yield have included it. The second best co-culture partner might be suggested to be *R. capsulatus* hup- (YO3), also single culture of which ranks the second. All four co-cultures including have ranked within top five.

R. palustris (DSM 127), although being the most successful bacterial strain in single culture experiments and co-culture combinations when it was together with *R. palustris*

(DSM 127), yields to lower substrate conversion efficiencies with the co-cultures of two different strains of PNS bacteria it forms with *R. capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) than *R. sphaeroides* O.U.001 (DSM 5864). Hence it might be suggested to be the third best bacterial strain as a co-culture partner.

R. capsulatus (DSM 1710) was the least efficient single culture bacterial strain and the partner of the least efficient co-cultures. Considering that *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) co-culture actually ranked below *R. palustris* (DSM 127), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) co-culture leads to lower yield than single culture of *R. palustris* (DSM 127) alone.

Different PNS bacteria exhibit differently on different substrates: The efficiencies of different strains are not the same for different substrates. Also the strains with highest hydrogen yields vary among substrate types. For instance, while the performance of *Rhodobacter* species dominate environments containing high levels of lower fatty acids, whereas *Rhodopseudomonas* species dominate environments containing low levels of lower fatty acids (Okubo et al., 2007).

In the literature, there are co-culture studies regarding PNS bacteria, which are conducted on different substrates, and have resulted in different results. The comparison of their results with this study implies that a composition of the feed is a very important factor for improved hydrogen yields of the co-cultures over single cultures.

For instance, Sasikala et al., (1994) have co-cultivated *R. sphaeroides* O.U. 001 with Pseudomonas *fluorescens* in free and immobilized forms on BP medium with 1% (w/v) glucose addition, and compared these co-cultures with single cultures of the same

strains. They have obtained a nearly 4-fold increase in comparison with single culture *R. Sphaeroides*, and more than 26-fold increase when compared with *Pseudomonas fluorescens*. Their results regarding immobilized cultures were similar: They have obtained more than 1.4-fold increase in comparison with single culture *R. Sphaeroides*, and about 1843-fold increase when compared with *Pseudomonas fluorescens*.

Kondo et al., (2002) have co-cultivated *R. Sphaeroides* RV and its reduced pigment mutant MTP4 in double layered reactor setup on basal medium containing 50 mM lactate, 10 mM glutamate and 18 mM hydrogen carbonate. They have obtained 1.33 fold higher hydrogen production than single culture *R. Sphaeroides* RV and 1.09 higher hydrogen production than *R. Sphaeroides* MTP4.

Sağır, (2012) has co-cultivated *R. capsulatus* YO3 (Hup-) and *R. palustris* (DSM 127), *R. sphaeroides O.U.001* (DSM 5864) *and R. palustris* (DSM 127), *R. capsulatus YO3 (Hup-)* and *R. sphaeroides* O.U.001 (DSM 5864), *and R. capsulatus* YO3 (Hup-), *R. sphaeroides* O.U.001 (DSM 5864) and *R. palustris* (DSM 127) together on molasses medium with 5 mM sucrose, and have compared the results with the single cultures of the same bacteria. For *R. capsulatus* YO3 (Hup-) and *R. palustris* (DSM 127), 1.13 fold increase was observed over *R. capsulatus* YO3 (Hup-) and 1.25 fold decrease was observed over *R. palustris* (DSM 127). Whereas for *R. sphaeroides O.U.001* (DSM 5864) *and R. palustris* (DSM 127), 2.16 fold increase was observed over *R. palustris* (DSM 127) and 2.86 fold increase was observed over *R. sphaeroides O.U.001* (DSM 5864). Also for *R. capsulatus* YO3 (Hup-) and *R. sphaeroides* O.U.001 (DSM 5864), 2.52 fold increase was observed over *R. capsulatus* YO3 (Hup-) and 2.37 fold increase was observed over *R. sphaeroides* O.U.001 (DSM 5864). And finally for for *R. capsulatus* YO3 (Hup-), *R. sphaeroides* O.U.001 (DSM 5864) and *R. palustris* (DSM

127), 2.60 fold increase was observed over for *R. capsulatus* YO3 (Hup-), 1.84 fold increase was observed over *R. palustris* (DSM 127), and 2.44 fold increase was observed over *R. sphaeroides* O.U.001 (DSM 5864).

In this study, generally slight increase in hydrogen yield was observed by the co-cultures of PNS bacteria, when compared with single cultures, in comparison with the studies by Sağır, (2012) and Sasikala, (1994) however generally higher hydrogen yields were observed in contrast with Kondo, (2002). Hence experimentations on a wider variety of substrates are required to acquire a good knowledge on benefits of co-cultivation of PNS bacteria on photofermentation.

A future prospect of this study would be conducting experiments on various carbon / nitrogen ratios, and comparing hydrogen yields of single cultures and co-cultures of PNS bacteria within fixed initial carbon / nitrogen ratios. Besides, when hydrogen production values of single cultures of PNS bacteria on growth medium were compared with single cultures of PNS bacteria on hydrogen production medium, different strains were found to exhibit better hydrogen production on different media. For instance, *R. sphaeroides* O.U.001 (DSM 5864) has led to the highest hydrogen production on growth medium, which was followed by *R. capsulatus* hup- (YO3) and *R. palustris* (DSM 127) respectively. However, on hydrogen production medium, *R. palustris* (DSM 127) has led to the highest hydrogen production, which was followed by *R. capsulatus* hup- (YO3) and *R. sphaeroides* O.U.001 (DSM 5864) respectively.

These results implicate the importance of determining hydrogen yields of co-cultures of PNS bacteria on different media with different carbon / nitrogen ratios. Additionally, light conversion efficiencies were found between 0.20 – 0.84 % for all single cultures

and co-cultures of PNS bacteria. Although these efficiencies appear to be low, they might be suggested to be consistent when compared with other studies (Sevinç, 2010, Sağır, 2012).

CHAPTER 4

4. CONCLUSIONS

In the present study co-cultures of two and three different strains of bacteria were cultivated on 40 mM Acetate / 2 mM Glutamate medium, and their hydrogen production capacities were compared with single cultures of these bacteria strains.
Based on the results obtained from experiments, these followings were deduced:

a) *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) produced maximum total hydrogen, 1.77±0.08 L / L_c, which also exhibited hydrogen yield of 2.23±0.10 mol H_2 / mol acetate.

b) *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) produced second maximum total hydrogen, 1.82±0.04 L / L_c, which has exhibited the highest hydrogen yield, 2.61±0.06 mol H_2 / mol acetate.

c) Co-cultivation of three different strains of PNS bacteria have yielded maximum hydrogen yield and these co-cultures could be suggested to improve hydrogen yield.

d) Co-cultivation of *R. palustris* (DSM 127) and *R. sphaeroides* O.U.001 (DSM 5864) has yielded the third highest hydrogen yield and this co-culture could be suggested to improve hydrogen yield.

e) Co-cultivation of *R. capsulatus* hup- (YO3) and *R. sphaeroides* O.U.001 (DSM 5864) have yielded higher hydrogen yield than single cultures of *R. capsulatus* hup- (YO3) and *R. sphaeroides* O.U.001 (DSM 5864), and this co-culture could be suggested to improve hydrogen yield.

f) The PNS bacterium which its co-cultures with other PNS bacteria have led to highest hydrogen yields was *R. sphaeroides* O.U.001 (DSM 5864), which was followed by *R. capsulatus* hup- (YO3) and *R. palustris* (DSM 127).

g) The co-cultures of *R. capsulatus* (DSM 1710) have led to least hydrogen yields, in some cases even lower than the single cultures of its partner.

h) Co-cultures including *R. capsulatus* (DSM 1710) or *R. capsulatus* hup- (YO3) exhibit instant growth similarly to the single cultures of the *R. capsulatus* (DSM 1710) and *R. capsulatus* hup- (YO3).

i) Due to faster accumulation of bacterial biomass, hydrogen productions have started faster for single culture and co-cultures of *R. capsulatus* (DSM 1710) or *R. capsulatus* hup- (YO3). Hence for processes which speed of hydrogen production is important, co-cultures including *R. capsulatus* (DSM 1710) or *R. capsulatus* hup- (YO3) may offer advantage.

j) For processes in which total amount of hydrogen is more important, co-cultures of *R. sphaeroides* O.U.001 (DSM 5864) and *R. palustris* (DSM 127)

may offer advantage.

k) Co-cultures including *R. capsulatus* (DSM 1710) or *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) together offer advantages of both speed and maximal hydrogen production.

l) In general, co-cultures gave better results, higher hydrogen productivities, hydrogen yields, and substrate conversion efficiencies, when compared with single cultures.

For further improvemets of hydrogen production by co-cultures of PNS bacteria, the following experiments have to be carried out:

a) Different experiments have to be conducted with co-cultures of PNS bacteria at a range of different temperatures between 10 °C – 45 °C.

b) Different experiments have to be conducted with different organic wastes and organic compounds having various compositions.

c) Experiments have to be conducted with immobilized co-cultures of PNS bacteria.

d) Absorption spectrum of different co-cultures of PNS bacteria would be useful for determining the most suitable light intensities to be utilized by different combinations of PNS bacteria co-cultures (Kondo, et al., 2002).

e) The composition of bacterial community has to be identified and dominant strains must be deduced.

REFERENCES

Akkerman, I., Janssen, M., Rocha, J., Wijffels, H.R. (2002). Photobiological hydrogen production: photochemical efficiency and bioreactor design. International Journal of Hydrogen Energy, 27, 1195-1208.

Akköse, S. (2008). Expression Analysis of nitrogenase Genes in *Rhodobacter sphaeroides* O.U.001 Grown under Different Physiological Conditions, Unpublished master's thesis, Middle East Technical University, Ankara, Turkey.

Akköse, S., Gündüz, U., Yücel, M., Eroğlu, İ. (2009). Effects of ammonium ion, acetate and aerobic conditions on hydrogen production and expression levels of nitrogenase genes in *Rhodobacter sphaeroides* O.U.001. International Journal of Hydrogen Energy, 34(21), 8818-8827.

BACAS Report. (2006). Hydrogen as an Energy Carrier, Royal Belgian Academy Council of Applied Science.

Basak, N., Das, D. (2007). Microbial Biohydrogen Production by *Rhodobacter sphaeroides* O.U.001 in Photobioreactor. Proceedings of the World Congress on Engineering and Computer Science 2007, October 24-26, 2007, San Francisco, USA.

Bayrakçeken, A., Smirnova, A., Kitkamthorn, U., Aindow, M., Türker, L., Eroğlu, İ., Erkey, C. (2008). Pt-based electrocatalysts for polymer electrolyte membrane fuel cells prepared by supercritical deposition technique. Journal of Power Sources, 179(2), 532–540.

Biebl, H., Pfennig, N. (1981). Isolation of Members of the Family Rhodosprillaceae, In M.P. Starr, H. Stolp, H.G.. Trüper, A. Balows, H.G. Schlegel (Eds), The prokaryotes (pp. 267-273). New York: Springer-Verlag.

Boran, E. (2011). Process Development for Continuous Photofermentative Hydrogen Production, Unpublished master's thesis, Middle East Technical University, Ankara, Turkey.

Burns, R.C., Hardy, R. W. F. (1975). Nitrogen fixation in bacteria and higher plants. Molecular Biology, Biochemistry and Biophysics, 21, 1-189.

Chong, M.L., Sabaratnam, V., Shirai Y., Hassan, M.A., (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation, International Journal of Hydrogen Energy, 34, 4755-4762.

Claassen, P.A.M., De Vrije, T. (2006). Non-thermal production of pure hydrogen from biomass: HYVOLUTION. International Journal of Hydrogen Energy, 31, 1416-1423.

Czuppon, T.A., Knez S.A., Newsome, D.S. (1996). Othmer Encyclopedia of Chemical Technology, New York: Wiley.

Das, D., Veziroğlu T. N. (2001). Hydrogen Production by Biological Processes: A Survey of Literature. International Journal of Hydrogen Energy, 26, 13-28.

De Vrije, T., Mars, A.E., Budde, M.A.W., Lai, M.H., Dijkema, C., De Waard, P., Claassen, P.A.M. (2007). Glycolytic pathway and hydrogen yield studies of the extreme thermophile *Caldicellulosiruptor saccharolyticus*. Applied Microbiology and Biotechnology, 74, 1358-1367.

Drepper, T., Gross, S., Yakunin, A.F., Hallenbeck, P.C., Masepohl, B., Klipp, W. (2003). Role of GlnB and GlnK in ammonium control of both nitrogenase systems in the phototrophic bacterium Rhodobacter capsulatus. Microbiology, 149(8). 2203-2212.

Eroğlu, E., Eroğlu, İ., Gündüz, U., Yücel, M. (2008). Effect of clay pretreatment on photofermentative hydrogen production from olive mill wastewater. Bioresource Technology, 99(15), 6799-6808.

Eroğlu, E., Eroğlu, İ., Gündüz, U., Yücel, M. (2009). Comparison of physicochemical characteristics and photofermentative hydrogen production potential of wastewaters produced from different olive oil mills in Western-Anatolia, Turkey. Biomass & Bioenergy, 33(4), 706-711.

Eroğlu, E., Eroğlu, İ., Gündüz, U., Yücel, M. (2009). Treatment of olive mill wastewater by different physicochemical methods and utilization of their liquid effluents for biological hydrogen production. Biomass & Bioenergy, 33(4), 701-705.

Eroğlu, E., Gündüz, U., Yücel, M., Eroğlu, İ. (2010). Photosynthetic bacterial growth and productivity under continuous illumination or diurnal cycles with olive mill wastewater as feedstock. International Journal of Hydrogen Energy, 35(11), 5293-5300.

Fascetti, E., Daddario, E., Todini, O., Robertiello, A. (1998). Photosynthetic hydrogen evolution with volatile organic acids derived from the fermentation of source selected municipal solid wastes. International Journal of Hydrogen Energy, 23(9), 753-760.

Fedorov, A.S., Tsygankov, A.A., Rao, K.K., Hall, D.O. (1998). Hydrogen photoproduction by *Rhodobacter sphaeroides* immobilised on polyurethane foam. Biotechnology Letters, 20, 1007.

Fu, H., Burris, R.H. (1989). Ammonium Inhibition of Nitrogenase Activity in Herbaspirillum seropedicae, Journal of Bacteriology, 171, 3168-3175.

Gaffron, H., Rubin, J. (1992). Fermentative and Photochemical Production of Hydrogen by Algae. Journal of General Physiology, 26, 219-40.

Gall, A., Robert, B. (1999). Characterization of the Different Peripheral Light-Harvesting Complexes from High- and Low-Light Grown Cells from *Rhodopseudomonas palustris.* Biochemistry, 38(16), 5185-90.

Ghirardi, M.L., Posewitz, M.C., Maness, P.C., Dubini, A., Yu, J., Siebert, M. (2007). Hydrogenases and Hydrogen Photoproduction in Oxygenic Photosynthetic Organism. Annual Review of Plant Biology, 58, 71-91.

Hallenbeck P, Ghosh D (2009) Advances in fermentative biohydrogen production: the way forward? Trends in Biotechnology 27, 287–29

Holladay, J.D., Hu, J., King, D.L., Wang, Y. (2009). An overview of hydrogen production Technologies. Catalysis Today, 139, 244-260.

Holt, J.G., Stanley, J.T., Bryant, M.P., Pfennig, M.P. (1984). Bergey's Manual of Systematic Bacteriology. Baltimore: Williams and Wilkins.

Hustede, E., Steinbiichel, A., Schlegel, H.G. (1993). Relationship between the photoproduction of hydrogen and the accumulation of PHB in non-sulphur purple bacteria. Applied Microbiology and Biotechnology, 39, 87-93.

Imhoff, J.F., Truper, H.G., Pfennig, N. (1984). Rearrangement of the Species and Genera of the Phototrophic Purple Nonsulfur Bacteria. International Journal of Systematic Bacteriology, 34, 240-343.

Imhoff, F.J. (1995). Taxonomy and physiology of phototropic purple bacteria and green sulfur bacteria. Anoxygenic Photosynthetic Bacteria 2, 1-15.

Kapdan, I., Kargi, F. (2006). Biohydrogen production from waste materials. Enzyme and Microbial Technology, 28, 569-582.

Karel, S.F., Libicki, S.B., Robertson, C.R. (1985). The immobilization of whole cells-engineering principles. Chemical Engineering Science, 40(8), 1321-1354.

Kars, G., Gündüz, U., Rakhely, G., Yücel, M., Eroğlu, İ., Kovacs, K.L. (2008). Improved

hydrogen production by uptake hydrogenase deficient mutant strain of *Rhodobacter sphaeroides* O.U.001. International Journal of Hydrogen Energy, 33, 3056-3060.

Kars, G., Gündüz, U., Yücel, M., Rakhely, G., Kovacs, K. L., Eroğlu, İ. (2009). Evaluation of hydrogen production by *Rhodobacter sphaeroides* OU001 and its hupSL deficient mutant using acetate and malate as carbon sources. International Journal of Hydrogen Energy, 34, 2184-2190.

Kim, M.S., Baek, J.S., Lee, J.K. (2006). Comparison of H2 accumulation by Rhodobacter sphaeroides KD131 and its uptake hydrogenase and PHB synthase deficient mutant. International Journal of Hydrogen Energy, 31(1), 121-127.

Kondo, T., Arakawa, M., Wakayama, T., Miyake, J. (2002). Hydrogen production by combining two types of photosynthetic bacteria with different characteristics. International Journal of Hydrogen Energy, 27(11–12), 1303–1308.

Koku, H., Eroğlu, I., Gündüz, U., Yücel, M., Türker, L. (2002). Aspects of the Metabolism of Hydrogen Production by *Rhodobacter sphaeroides*. International Journal of Hydrogen Energy, 2, 1315-1329.

Korbas, M., Vogt, S., Meyer-Klaucke, W., Bill, E., Lyon, E.J., Thauer, R.K., Shima, S. (2006). The Iron-Sulfur Cluster-free Hydrogenase (Hmd) Is a Metalloenzyme with a Novel Iron Binding Motif. Journal of Biological Chemistry, 281(41), 30804-30813.

Kosher, S.A. (1948). Growth Factors for Microorganisms. Annual Review of Microbiology, 2, 121-142.

Kovacs, K.L., Kovacs, A.T., Maroti, G., Bagi, Z., Csanadi, G., Perei, K., Balint, D. (2004). Improvement of biohydrogen production and intensification of biogas formation. Reviews in Environmental Science and Bio/Technology, 3, 321-330

Lang, FS., and Oesterhelt, D. (1989). Microaerophilic growth and induction of the

photosynthetic reaction center in Rhodopseudomonas viridis. Journal of Bacteriology, 171(5), 2827–2834.

Masepohl, B., Drepper, T., Klipp, W. (2004). Genetics and regulation of nitrogen fixation in free-living bacteria. In Klipp, W., Masepohl, B., Gallon, J.R., Newton, W.E. (eds), Kulwer Academic Publishers, Netherlands, pp. 141-173.

Masepohl, B., Hallenback, P.C. (2010). Nitrogen and Molybdenum Control of Nitrogen Fixation in the Phototrophic Bacterium *Rhodobacter capsulatus*. Advances in Experimental Medicine and Biology, 675(2), 49-70.

Mc, Auliffe, C. A. (1980). Hydrogen and Energy. London: The Macmillan Press.

McGrath, J.E., Harfoot, C.G. (1997). Reductive dehalogenation of halocarboxylic acids by the phototrophic genera Rhodospirillum and Rhodopseudomonas. Applied and Environmental Biology, 63(8), 3333-3335.

Nandi, R., Sengupta, S. (1998). Microbial production of hydrogen: an overview. Critical Reviews in Microbiology, 24(1), 61-84.

Okubo, Y., Hirishi, A. (2007). Population Dynamics and Acetate Utilization Kinetics of Two Different Species of Phototrophic Purple Nonsulfur Bacteria in a Continuous Co-culture System. Microbes and Environments, 22(1), 82-87.

Özgür, E., Afsar, N., Vrije, T., Yücel, M., Gündüz, U., Claassen, P.A.M., Eroğlu, İ. (2010). Potential use of thermophilic dark fermentation effluents in photofermentative hydrogen production by Rhodobacter capsulatus. Journal of Cleaner Production, 18(1), 23-28.

Özgür, E., Mars, A.E., Peksel, B., Louwerse, A., Yücel, M., Gündüz, U., Classen, P.A.M., Eroğlu, İ. (2010). Biohydrogen production from beet molasses by sequential dark and photofermentation. International Journal of Hydrogen Energy, 35(2), 511-517.

Özgür, E., Uyar, B., Öztürk, Y., Yücel, M., Gündüz, U., Eroğlu, İ. (2010). Biohydrogen production by *Rhodobacter capsulatus* on acetate at fluctuating temperatures, Resources Conservation and Recycling, 54(5), 310-314.

Öztürk, Y. (2005). Characterisation of the Genetically Modified Cytochrome Systems and Their Application to Biohydrogen Production in *Rhodobacter Capsulatus*. PhD thesis in Biotechnology. Middle East Technical University, Ankara.

Öztürk, Y., Yücel, M., Daldal, F., Mandacı, S., Gündüz, U., Türker, L., Eroğlu, İ. (2006). Hydrogen production by using *Rhodobacter capsulatus* mutants with genetically modified electron transfer chains. International Journal of Hydrogen Energy, 31, 1545-1552.

Pellerin, N.B., Gest, H. (1983). Diagnostic Features of the Photosynthetic Bacterium Rhodopseudomonas sphaeroides. Current Microbiology, 9, 339-344.

Pierrard, J., Ludden, P.W., Roberts, G.P. (1993). Posttranslational Regulation of Nitrogenase in *Rhodobacter capsulatus*: Existence of Two Independent Regulatory Effects of Ammonium, Journal of Bacteriology. 175, 1358-1366.

Ramachandran, R., Menon, R.K. (1998). An Overview of Industrial Uses of Hydrogen, International Journal of Hydrogen Energy. 23, 593-8.

Richmond, A., Zou, N. (1999). Effect of Light-path Length in Outdoor Flat Plate Reactors on Output Rate of cell mass and of EPA in *Nannochloropsis sp*. Journal of Biotechnology, 70, 351-356.

Riis, T., Hagen, E.F., Vie, P.J.S., Ulleberg, Ø. (2005). Hydrogen Production – Gaps and Priorities IEA Hydrogen Implementing Agreement (HIA), 11 pages.

Sağır, E. (2012). Photobiological Hydrogen Production from Sugar Beet Molasses. Unpublished master's thesis, Middle East Technical University, Ankara, Turkey.

Sanchezi C.G., Rüdiger, O., Fernandez, V.M., De Lacey, A.L., Marques, M., Pereira, I.A.C. (2010). Interaction of the active site of the Ni–Fe–Se hydrogenase from Desulfovibrio vulgaris Hildenborough with carbon monoxide and oxygen inhibitors. JBIC Journal of Biological Inorganic Chemistry, 15(8), 1285-92.

Sari, S. (2007). Development of helical tubular reactor for hydrogen producing photosynthetic bacteria, Unpublished master's thesis, Middle East Technical University, Ankara, Turkey.

Sasikala, K., Ramana, C.V., Rao, P.R., 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, International Journal of Hydrogen Energy, 15, 795-797.

Sasikala, K., Ramana, C.H.., Rao, P.R. (1991). Environmental Regulation for Optimal Biomass Yield and Photoproduction of Hydrogen by *Rhodobacter sphaeroides* O.U.001. International Journal of Hydrogen Energy, 16, 597-601.

Sasikala, K., Ramana, C.V., Rao, PR.., Kovacs, K.L. (1993). Anoygenic Phototropic Bacteria: Physiology and Advances in Hydrogen Production Technology. Advanced Applied Microbiology, 38, 211-295.

Sasikala, Ch., Ramana, Ch.V., Prasad, G.S. (1994). H2 production by mixed cultures, World Journal of Microbiology & Biotechnology, 10, 221-223.

Sevinç, P. (2010). Kinetic analyses of the effects of temperature and light intensity on growth, hydrogen production and organic acid utilization by *Rhodobacter capsulatus*. Unpublished master's thesis, Middle East Technical University, Ankara, Turkey.

Shima, S., Thauer, R.K. (2007). A third type of hydrogenase catalyzing H2 activation. The Chemical Record, 7(1), 37-46.

Singh, A., Misra, K. (2009), Improvement of Hydrogen Production by Immobilized Rhodopseudomonas palustris CGA009 Using Reverse Micelles as Microreactor. In Nanotechnology 2009: Biofuels, Renewable Energy, Coatings, Fluidics and Compact Modeling. Cambridge, CRC Press.

Staley, J.T., Brenner, D.J., Krieg, N.R. (2005). Bergey's Manual® of Systematic Bacteriology: Volume Two: The Proteobacteria, Springer.

Şengül, E., Erdener, H., Akaya, R.G., Yücel, H., Baç, N., Eroğlu, İ. (2009). Effects of sulfonated polyether-etherketone (SPEEK) and composite membranes on the proton exchange membrane fuel cell (PEMFC) performance. International Journal of Hydrogen Energy, 34(10), 4645-4652.

Tredici, M.R., Zitelli, G. (1997). Cultivation of *Spirulina (Arthrospira)* in Flat Plate Reactors, In *Spirulina platensis(Arthrospira)*: Physiology, Cell-biology and Biotechnology. Avigad Vonshak (Ed.), Taylor and Francis, 117-130.

Tsygankov, A.A., Hirata, Y., Asada, Y., Miyake, J. (1993). Immobilization of the purple non-sulfur bacterium Rhodobacter sphaeroides on glass surfaces. Biotechnology Techniques, 7(4), 283-286.

Uyar, B. (2008). Hydrogen Production by Microorganisms in Solar Bioreactor. Unpublished doctoral dissertation, Middle East Technical University, Ankara, Turkey.

Uyar B., Eroğlu, İ., Yücel, M., Gündüz, U. (2008). Photofermentative hydrogen production from volatile fatty acids present in dark fermentation effluents. International Journal of Hydrogen Energy, 34(10), 4517–4523.

Veziroğlu, T.N. (1995). Twenty Years of the Hydrogen Movement 1974 - 1994, International Journal of Hydrogen Energy 20, 1-7.

Vignais, P.M., Colbeau, A., Willison, J.C., Jouanneau, Y. (1985). Hydrogenase, Nitrogenase, and Hydrogen Metabolism in Photosynthetic Bacteria. Advance in Microbial Physiology, 26, 154-234.

Vignais, P.M., Billoud, B. (2007). Occurrence, Classification, and Biological Function of Hydrogenases: An Overview. Chemical Reviews, 107(10), 4206-4272.

Web site of the Energy Efficiency and Renewable Energy Network of the U.S. Department of Energy: www.eren.doe.gov/hydrogen, Last accessed on 04.09.2012.

Web site of the Forth Institute of Molecular Biology and Biotechnology, Rhodobacter Capsulatus.

http://ecoserver.imbb.forth.gr/microbiology/IMAGES/Rhodobacter_capsulatus.jpg. Last accessed on 04.09.2012

Website of HYVOLUTION, http://www.biohydrogen.nl. Last accessed on 04.09.2012.

Whittenbury, R., McLee, A.G. (1967). Rhodopseudomunas palustris and Rh. viridis--photosynthetic budding bacteria, Archives of Mikrobiology, 59(1), 324–334.

Web site of the Kenyon College, Microbe Wiki, Rhodobacter, http://microbewiki.kenyon.edu/index.php/Rhodobacter. Last accessed on 04.09.2012.

Yu, J., Takahashi, P. (2007). Biophotolysis-based hydrogen production by Cyanobacteria and green microalgae. In A. Mendez-Vilas (Ed.), Communicating Current Research and Educational Topics and Trends in Applied Microbiology (pp. 79-89). Badajoz, Spain: Formatex Publishing.

APPENDIX A

COMPOSITIONS OF MEDIA, VITAMINS, TRACE ELEMENT AND Fe-CITRATE SOLUTIONS

Table A.1 The composition of 1 liter of growth and hydrogen production medium

Medium Composition	Growth Medium	Hydrogen Production Medium
KH_2PO_4	3 g	3 g
$MgSO_4.7H_2O$	0.5 g	0.5 g
$CaCl_2.2H_2O$	0.05 g	0.05 g
Na-Acetate	1.15 ml	2.29 ml
Na-Glutamate	1.85 g	0.36 g
Vitamin Solution	0.1 ml	0.1 ml
Trace Element Solution	0.1 ml	0.1 ml
Fe-Citrate	0.5 ml	0.5 ml

Table A.2 The composition of 100 mL of vitamin solution (10 x)

Composition	Amount
Thiamine chloride hydrochloride	500 mg
Niacin (Nicotinic Acid)	500 mg
D+ Biotin	15 mg

Table A.3 The composition of 100 mL of trace element solution (10 x)

Composition	Amount
$ZnCl_2$	70 mg
$MnCl_24.H_2O$	100 mg
H_3BO_3	60 mg
$CoCl_2.6H_2O$	200 mg
$CuCl_2.2H_2O$	20 mg
$NiCl_2.6H_2O$	20 mg
$Na_2MoO_4.2H2O$	40 mg
HCl (25% V/V)	1 mg

The composition of 100 mL of Fe-citrate solution (50 x):

5 g Fe-citrate was dissolved in 100 mL distilled water and autoclaved for sterilization.

APPENDIX B

GROWTH PROFILE - DRY CELL WEIGHT CALIBRATION CURVES

B1. Dry Cell Weight Determination of PNS bacteria

Dry cell weights were calculated according to the calibration curves below:

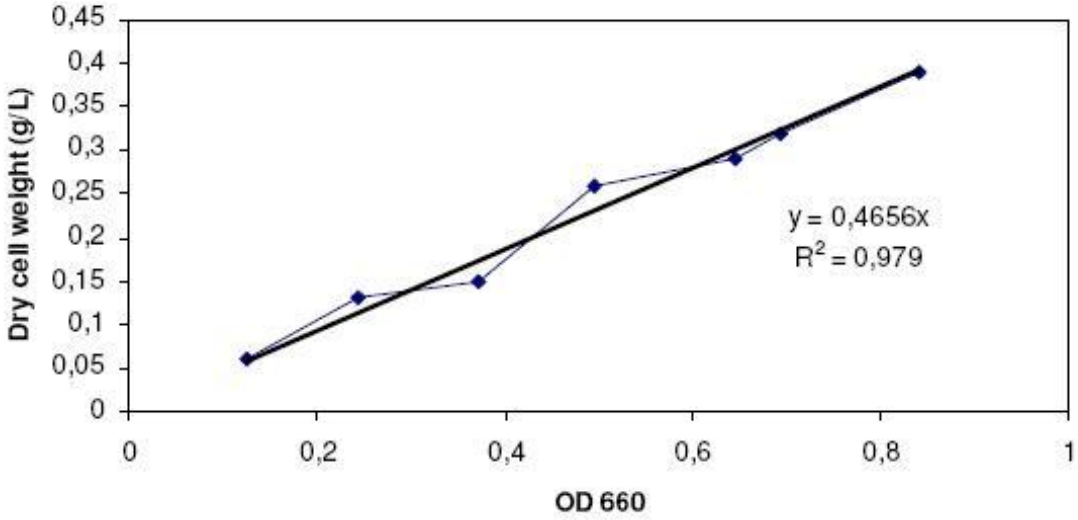


Figure B.1 Calibration curve and the regression trend line for *R. Capsulatus* YO3 dry weight versus absorbance at 660 nm (Öztürk, 2005).

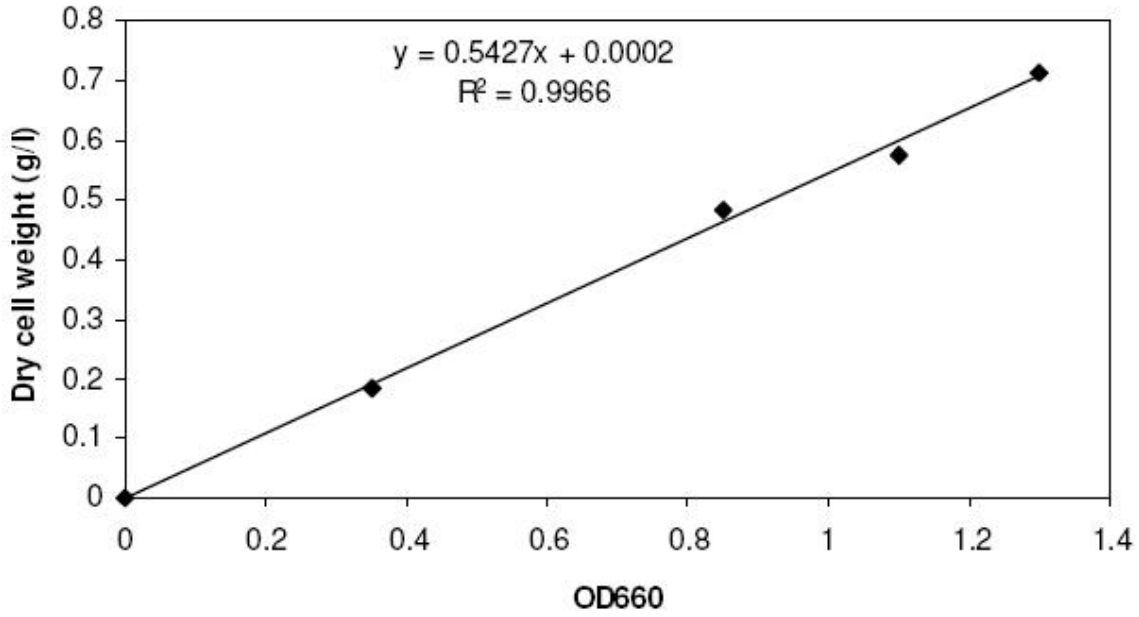


Figure B.2 Calibration curve and the regression trend line for *R. capsulatus* (DSM 1710) dry weight versus absorbance at 660nm (Uyar, 2008).

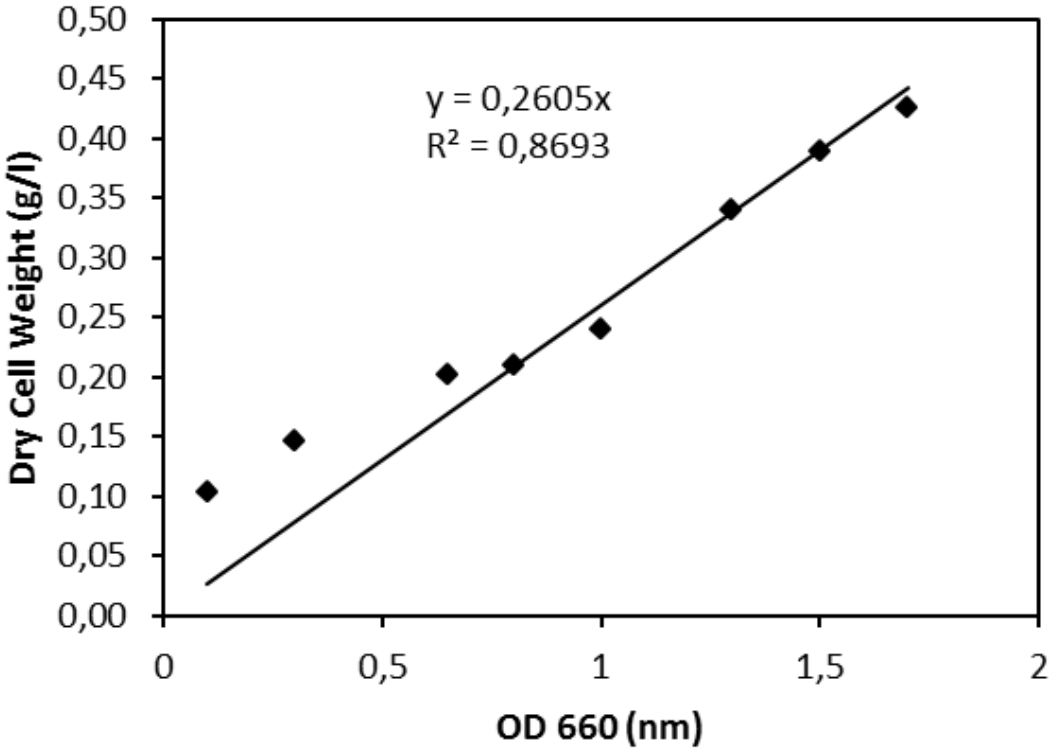


Figure B.3 Calibration curve and the regression trend line for *R. palustris* (DSM 127) dry weight versus absorbance at 660nm.

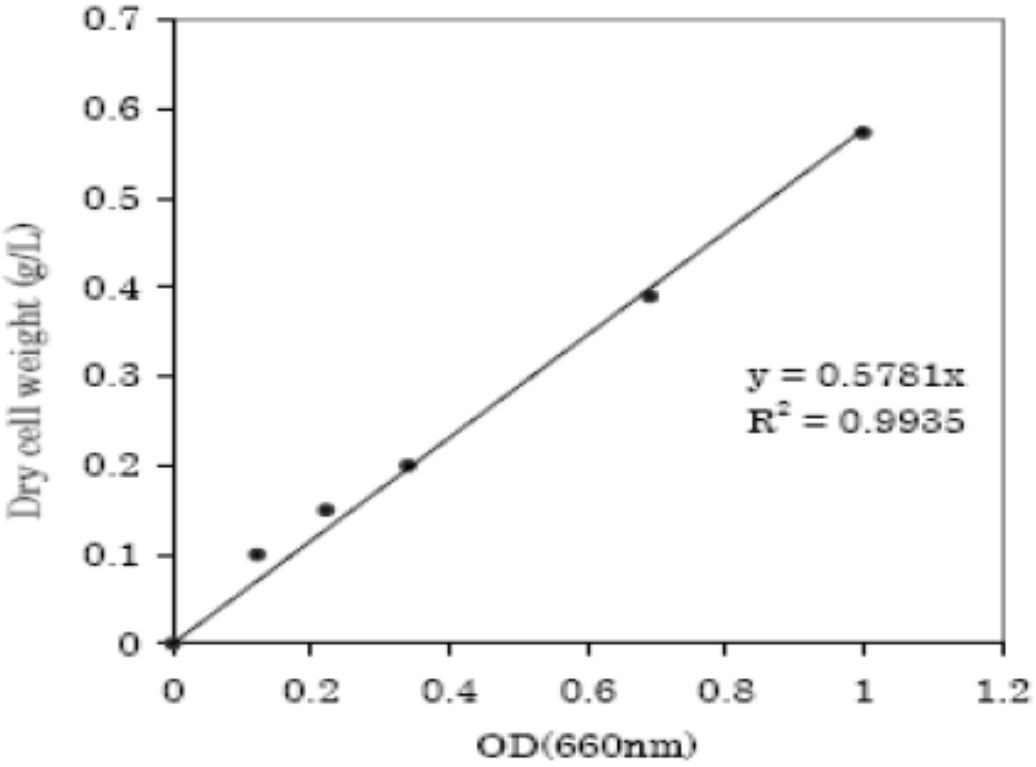


Figure B.4 Calibration curve and regression trend line for *R. sphaeroides* OU001 dry weight versus absorbance at 660nm (Eroğlu, 2006).

B.2 Biomasses of PNS Bacteria

If biomass growths were compared, *R. sphaeroides* O.U.001 (DSM 5864) has shown the highest biomass growth. *R. capsulatus* hup- (YO3) was the bacterial strain which has exhibited the second highest growth, and *R. palustris* (DSM 127) was the bacterial strain which has exhibited the lowest biomass growth. This result applies to both final biomass growths. Initially the biomass growth of *R. capsulatus* hup- (YO3) was higher than the

biomass growth of *R. sphaeroides* O.U.001 (DSM 5864), but the biomass growth of *R. sphaeroides* O.U.001 (DSM 5864) has exceeded the biomass growth of *R. capsulatus* hup- (YO3) between 24th and 48th hours.

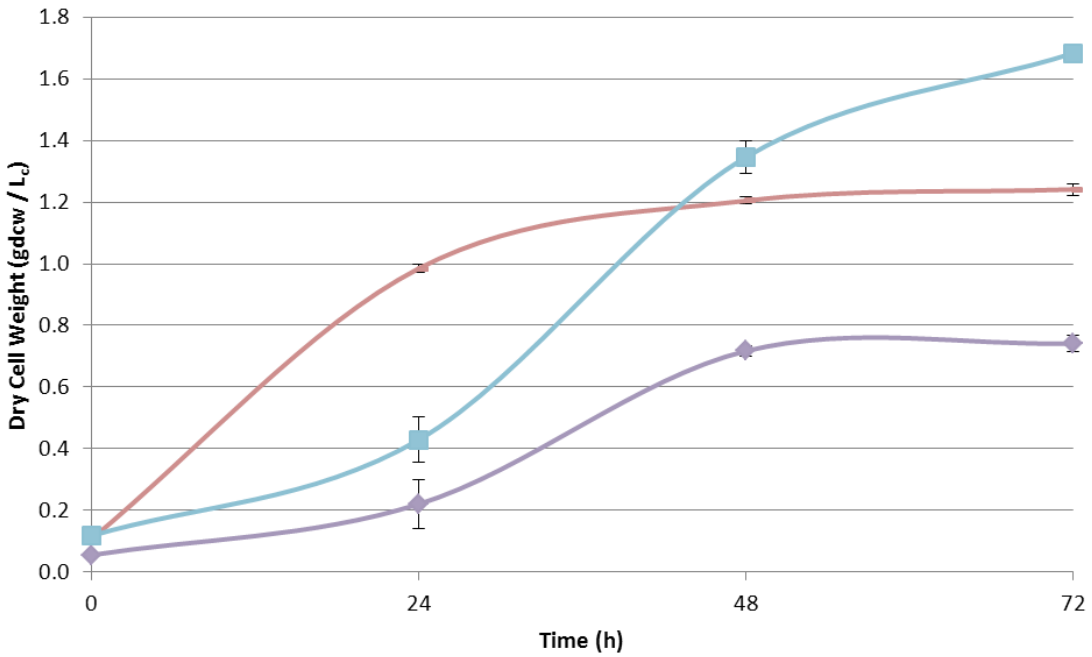


Figure B.5: Biomass accumulation values of *R. capsulatus* hup- (YO3), *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on 20 mM Acetate / 10 mM Glutamate BP Medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Biomass accumulations of *R. capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) were very close and the highest for the first 24 hours. Between the 24th hour, biomass accumulations of *R. capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) had decreased, but still their biomass was higher than other groups. At the 96th hour, their

biomasses peaked, after which began to decrease. The biomasses of R. *capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) were observed somewhat parallel to each other during the experimentation. *R. sphaeroides* O.U.001 (DSM 5864) has exhibited lower biomass accumulation than *R. capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) during the first 24 hours, but the biomass accumulation exponentially increased at every new 24 hour period until 72th hour. The biomass accumulation of the *R. palustris* (DSM 127) was the lowest throughout the experimentation; however its biomass accumulation also increased exponentially for every new 24 hour period until 72th hour. The biomass of *R. sphaeroides* O.U.001 (DSM 5864) has exceeded the biomasses of *R. capsulatus* hup- (YO3) and *R. capsulatus* (DSM 1710) between 48th and 72th hours and peaked at 168th hour, after which, the biomass of *R. sphaeroides* O.U.001 (DSM 5864) began to decrease. The biomass of *R. palustris* (DSM 127) has peaked at the 120th hour, after which it has preserved its biomass. (Figure B.6)

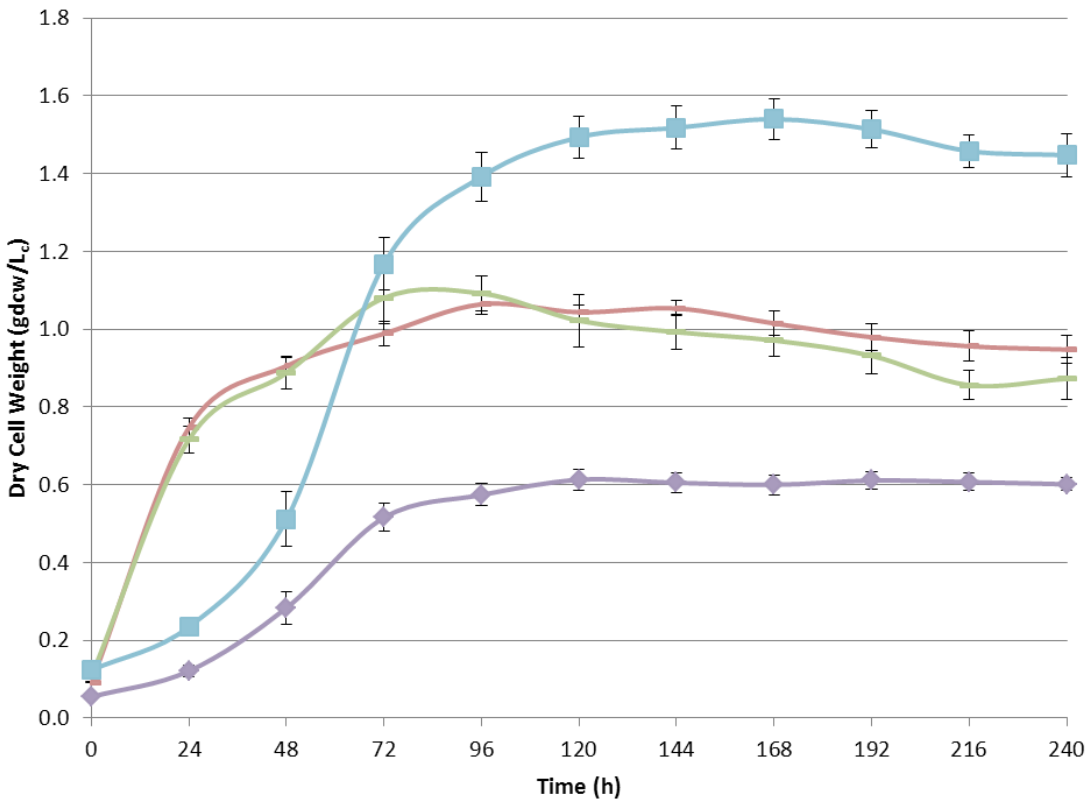


Figure B.6: Biomass accumulation values of *R. capsulatus* hup- (YO3), *R. capsulatus* (DSM 1710) , *R. palustris* (DSM 127), and *R. sphaeroides* O.U.001 (DSM 5864) cultured on 40 mM Acetate / 2 mM Glutamate BP Medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Biomass accumulations of *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864) and *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) were the very close and the highest during the first 24 hour period. Biomass accumulations of *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) and *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) were very close to each other and has followed the biomass

accumulations of *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864) and *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864). After the 24th hour, biomass accumulations of *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) and *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) began to decrease gradually for every new 24 hour period. Between the 24th hour and the 48th hour, the biomass of *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864) has exceeded the biomass of *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864). The biomasses of *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864) and *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) were higher than other groups throughout the experiment, and have peaked at the 120th hour. The biomasses of *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) and *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) were very close throughout the experiment, and have peaked at the 96th hour. The biomass accumulation of the *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) was lower than all other groups in the first 24 hours period. In the second 24 hours period, *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) group has preserved its biomass accumulation in the first 24 hours, and have reached the biomasses of *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) and *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) at the 48th hour. After 48th hour, the biomass of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) group was very close to *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) and *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) groups, but somewhat a bit higher. (Figure B.7)

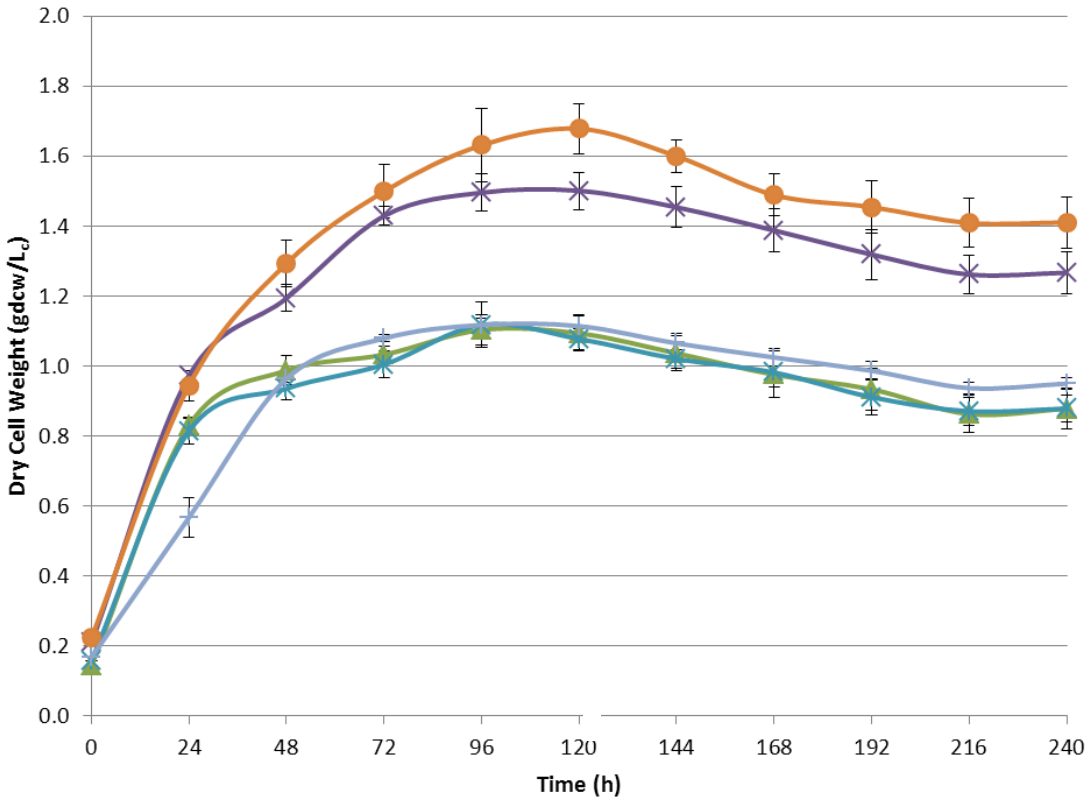


Figure B.7: Biomass accumulation values of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864), *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127), *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on 40 mM Acetate / 2 mM Glutamate BP Medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Biomasses of two triple co-culture groups were similar throughout the experimentation. Both groups had their highest biomass accumulations during the first 24 hour period,

after which biomass accumulations were slowed down. Maximum biomass was observed at the 120th hour for *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) *R. sphaeroides* O.U.001 (DSM 5864) group, and at the 96th hour for *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) *R. sphaeroides* O.U.001 (DSM 5864) group. Throughout the experiment, the biomass of the *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) *R. sphaeroides* O.U.001 (DSM 5864) group was a bit higher than the *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) *R. sphaeroides* O.U.001 (DSM 5864) group. (Figure B.8)

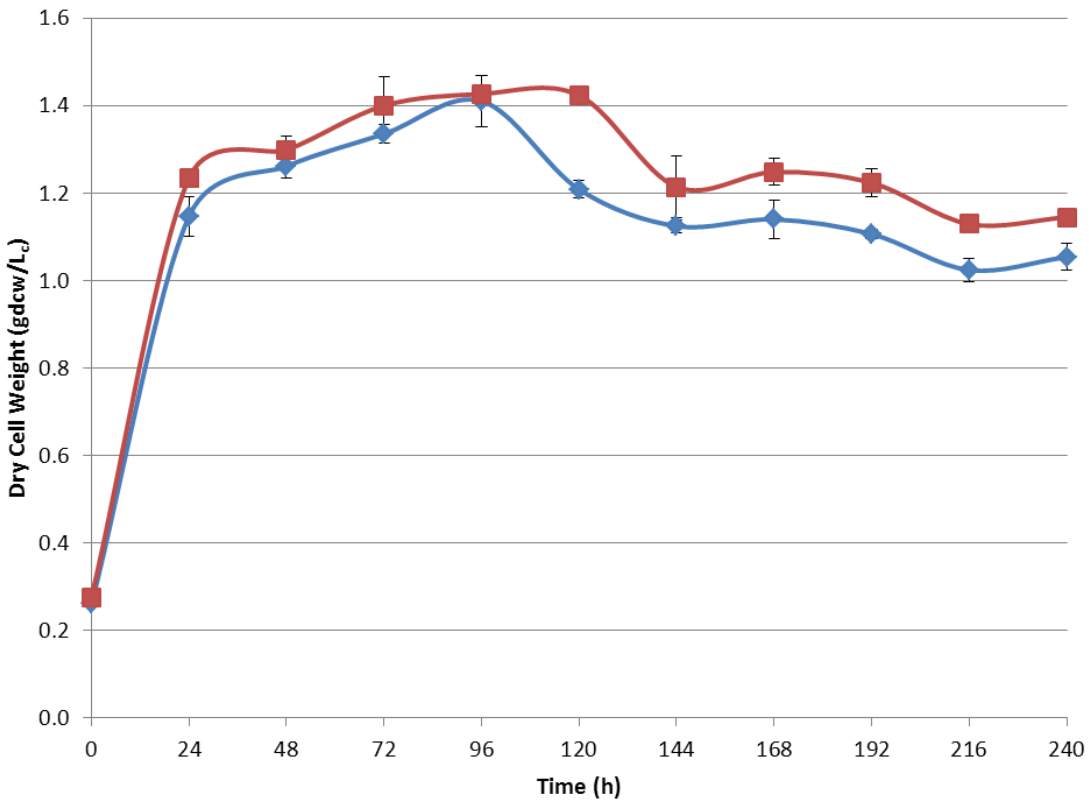


Figure B.8: Biomass accumulation values of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864), and *R. capsulatus* (DSM 1710) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on 40 mM Acetate / 2 mM Glutamate BP Medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

When the biomass growths of single cultures, double co-cultures, and triple co-cultures were compared together, although initially triple co-culture groups have exhibited highest biomass growth, the highest biomass growth in total was observed for *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864), which was followed by

R. sphaeroides O.U.001 (DSM 5864), *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) and triple co-culture groups respectively.

Hence all experimented groups including *R. sphaeroides* O.U.001 (DSM 5864) but lacking *R. palustris* (DSM 127) were superior to other ones in means of biomass growth, and even co-cultivation did not bring advantage to groups including *R. sphaeroides* O.U.001 (DSM 5864) except the *R. capsulatus* (DSM 1710) + *R. sphaeroides* O.U.001 (DSM 5864) group, which has exhibited the highest biomass growth, when considering the biomass growth of the single culture *R. sphaeroides* O.U.001 (DSM 5864) bacterial strain ranking the second.

Single culture *R. palustris* (DSM 127) bacterial strain has exhibited the lowest biomass growth throughout the experiment, and all the remaining groups, including double cultures including *R. palustris* (DSM 127), and single culture R. *capsulatus* hup- (YO3) and R. *capsulatus* (DSM 1710) groups have exhibited very similar biomass growth throughout the experiment, which was higher than the single culture *R. palustris* (DSM 127), but lower than the groups including *R. sphaeroides* O.U.001 (DSM 5864) but lacking *R. palustris* (DSM 127) in total.

APPENDIX C

ORGANIC ACID ANALYSIS

C.1 Sample Acetate HPLC Chromatogram

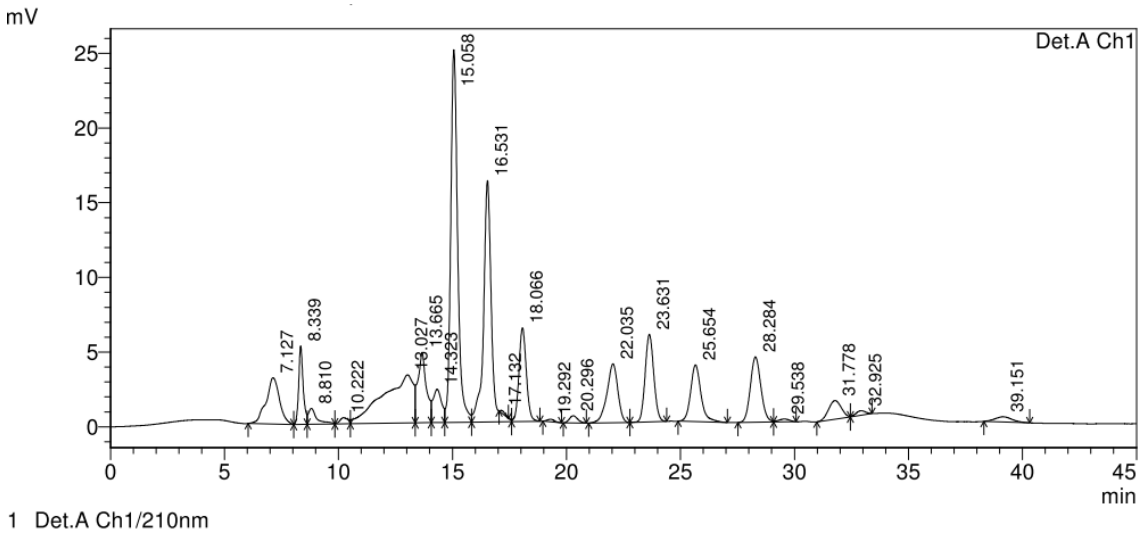


Figure C.1 Sample HPLC organic acid chromatogram (Acetate peak in 25.654 min, Shimadzu Agilent 10A series HPLC, UV 210 nm detector).

C.2 Acetate HPLC Calibration Curve

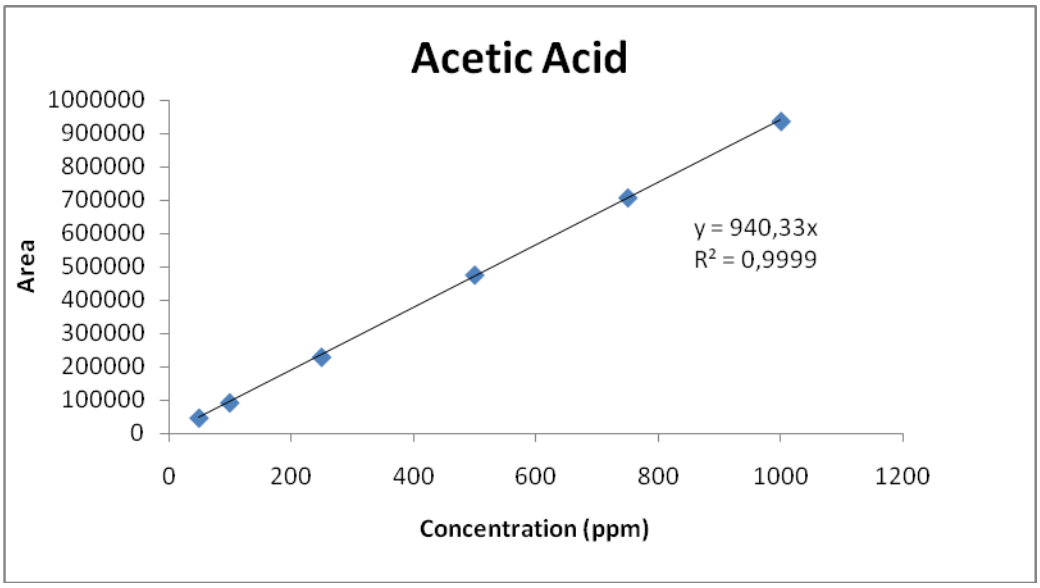
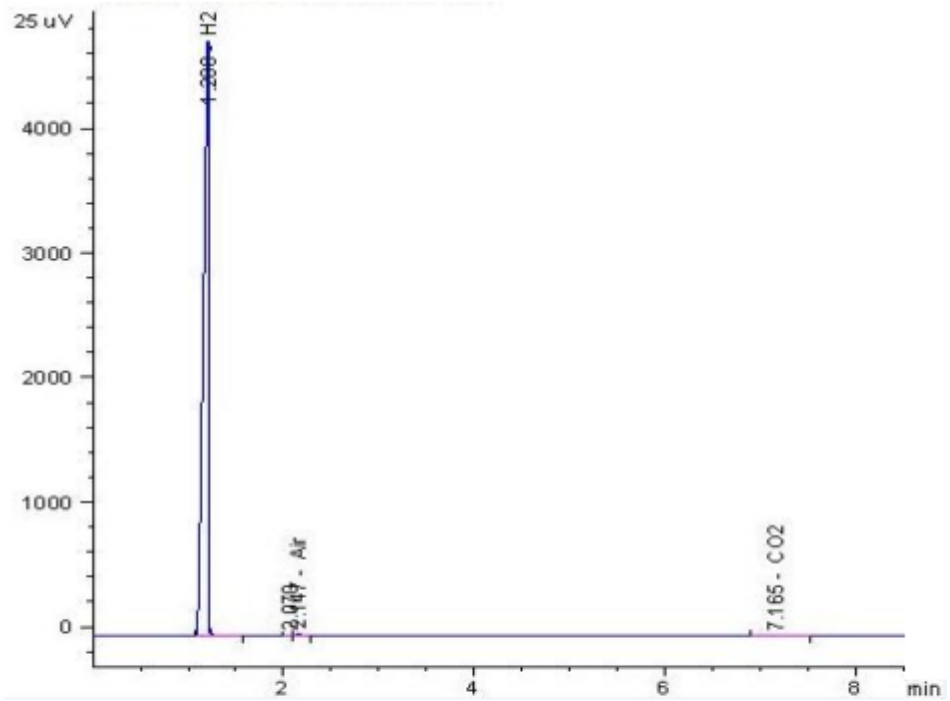


Figure C.2 Sample curve of sample acetate.

APPENDIX D

SAMPLE GAS CHROMATOGRAM



RetTime [min]	Type	Area [25 uV*s]	Amt/Area	Norm %	Grp	Name
1.208	BB	1.78157e4	5.36760e-3	92.790983		H2
2.147	VB	104.16668	4.30476e-2	4.351104		Air
7.165	BB	52.88443	5.56929e-2	2.857913		CO2

Figure D. Sample gas chromatogram (Agilent Technologies 6890 N gas chromatography) (Androga, 2009),

APPENDIX E

HYDROGEN YIELD CALCULATION

$$\text{Hydrogen Yield} = \frac{\text{Mass of hydrogen produced (g)}}{\text{Mass of substrates utilized}}$$

A sample substrate conversion efficiency (yield) calculation for the 40 mM/2 mM Ac/Glu fed *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864).

$$= \frac{2 \text{ x } 1.00794 \text{ (g / mol) x } 272.8 \text{ (mL) x } 0.0000402021259336505 \text{ (mol / mL)}}{0.105 \text{ (L) x } 0{,}040 \text{ (mol) x } 60.05 \text{ (g / mol)}}$$

APPENDIX F

SUBSTRATE CONVERSION EFFICIENCY CALCULATION

$$\text{Substrate conversion efficiency} = \frac{\text{Actual moles of } H_2 \text{ produced}}{\text{Theoretical moles of } H_2 \text{ produced}} \times 100$$

$$= \frac{\text{moles of } H_2 \text{ produced}}{4 \times \text{moles of Acetate Utilized}}$$

A sample substrate conversion efficiency (yield) calculation for the 40 mM/2 mM Ac/Glu fed *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864).

$$= \frac{0.2728 \text{ L} \times 0.0000402021559336505 \text{ g/mol}}{0.105 \text{ L} \times 4.40 \text{ g/mol}} \times 100 = 65.28\%$$

APPENDIX G

HYDROGEN PRODUCTIVITY CALCULATION

Calculation of hydrogen productivity

A sample hydrogen productivity calculation for the 40 mM/2 mM Ac/Glu fed *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864).

t = Duration of hydrogen production (hour) = 96 h
v = Volume of culture = 0.15 l
V_{H2} = Produced hydrogen = 10.97 mmol

Hydrogen productivity (mmol H_2/L_c.h)
= 10.97 mmol / (0.15 L x 96 h) = 0.76 mmol / L x h

APPENDIX H

LIGHT CONVERSION EFFICIENCY CALCULATION

Calculation of light conversion efficiency

A sample light conversion efficiency calculation for the 40 mM/2 mM Ac/Glu fed *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864).

V_{H2} = 0.2728 l
I = 114.28 W/m^2
d_{H2} = 0.089 g/L
A = 0.011 m^2
t = Duration of H_2 production

Light conversion efficiency (%) = [(33.6 x d_{H2} x V_{H2}) / (I x A x t)] x 100
= [(33.6 x 0.089 g/L x 0.2728 L) / (114.28 W/m^2 x 0.011 m^2 x 96 h)] x 100 = 0.68%

I. EXPERIMENTAL DATA

Table I.1 Total hydrogen production of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1S2**	**C2S2**	**C1S3**	**C2S3**
0	0	0	0	0
24	10	10.5	10.5	12
48	21.5	22.5	22.5	24
72	24.4	24.25	26	28

Table I.2 Total hydrogen production of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1S1**	**P2S1**	**P1S2**	**P2S2**	**P1S3**	**P2S3**
0	0	0	0	0	0	0
24	8	9	3.25	2.75	2.5	2.5
48	13	14	14.25	12.75	13.5	14.5
72	13	14	15.5	13.5	13.8	15.1

Table I.3 Total hydrogen production of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1S1**	**S2S1**	**S1S2**	**S2S2**	**S1S3**	**S2S3**	**S1S4**	**S2S4**
0	0	0	0	0	0	0	0	0
24	5	5	4.5	5.5	9	7	6	6
48	15	16	17.5	18.5	23	21	19	19
72	28	30	31.5	33.5	25	23.3	25	26

Table I.4 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1S2**	**C2S2**	**C1S3**	**C2S3**	**C1S4**	**C2S4**
0	0.25	0.24	0.28	0.28	0.24	0.23
24	2.3	2.27	2.25	2.21	2.13	2.17
48	2.77	2.7	2.68	2.63	2.8	2.69
72	2.81	2.77	2.91	2.71	2.84	2.72

Table I.5 Absorbance values at 660nm measured with 1 / 10 dilution of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1S2**	**P2S2**	**P1S3**	**P2S3**
0	0.21	0.2	0.24	0.22
24	0.71	0.73	0.95	1.06
48	2.88	2.97	2.89	2.89
72	3.02	2.97	3.2	3.03

Table I.6 Absorbance values at 660nm measured with 1 / 10 dilution of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1S3**	**S2S3**	**S1S4**	**S2S4**
0	0.24	0.23	0.19	0.19
24	0.93	0.8	0.63	0.6
48	2.47	2.58	2.84	2.85
72	3.13	3.2	3.15	3.1

Table I.7 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus* hup-(YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1S2**	**C2S2**	**C1S3**	**C2S3**	**C1S4**	**C2S4**
0	0.21	0.208	0.206	0.214	0.208	0.212
24	2.046	1.946	1.994	2.026	1.994	2.038
48	2.432	2.43	2.478	2.526	2.468	2.434
72	2.564	2.524	2.546	2.542	2.55	2.5

Table I.8 Absorbance values at 660nm measured with 1 / 2 dilution of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1S2**	**P2S2**	**P1S3**	**P2S3**
0	0.21	0.204	0.216	0.21
24	0.7	0.72	0.906	0.994
48	2.536	2.602	2.626	2.626
72	2.598	2.662	2.658	2.638

Table I.9 Absorbance values at 660nm measured with 1 / 2 dilution of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1S3**	**S2S3**	**S1S4**	**S2S4**
0	0.2	0.206	0.19	0.19
24	0.928	0.79	0.652	0.61
48	2.24	2.34	1.706	1.594
72	2.708	2.766	2.638	2.594

Table I.10 pH values of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1S2**	**C2S2**	**C1S3**	**C2S3**
0	6.4	6.4	6.4	6.4
24	7.646	7.705	7.957	7.914
48	7.726	7.724	7.476	7.519
72	7.256	7.484	7.396	7.315

Table I.11 pH values of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1S2**	**P2S2**	**P1S3**	**P2S3**
0	6.4	6.4	6.4	6.4
24	6.726	6.733	6.79	6.83
48	7.518	7.918	7.659	7.669
72	7.793	7.975	7.759	7.656

Table I.12 pH values of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1S3**	**S2S3**	**S1S4**	**S2S4**
0	6.4	6.4	6.4	6.4
24	6.847	6.758	6.658	6.967
48	6.836	7.02	7.198	7.289
72	7.176	7.659	7.092	7.093
			7.366	7.242

Table I.13 Total hydrogen production of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPSA**	**CHPSB**	**CHPSC**	**CHPSD**	**CHPSE**
0	0	0	0	0	0
24	102	96	100	112	134.4
48	184	179.2	182.4	196.8	209.6
72	238	235.2	232	249.6	256
96	262	249.6	256	273.6	272
120	268	251.2	262	280	280
144	270	252.8	265	283.2	281.6
168	271	252.8	266.4	284	281.6
192	271.4	253.6	267	285.6	284.8
216	271.4	254.4	267	285.6	284.8
240	271.4	254.4	267	286.4	284.8

Table I.14 Total hydrogen production of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPSA**	**CWPSB**	**CWPSC**	**CWPSD**	**CWPSE**
0	0	0	0	0	0
24	78.4	88.8	74	85.6	105.6
48	158.4	168.8	144.8	160	200
72	212.8	222.4	213.4	208	244
96	240	250.4	260	209.6	245.6
120	254.4	260	274.4	230.4	248.8
144	256.8	262.4	276.8	246.4	252
168	257.6	262.4	278.4	249.6	252
192	259.2	262.4	278.4	250.4	252.8
216	260.8	262.4	278.4	251.2	252
240	260.8	262.4	278.4	251.2	253.6

Table I.15 Total hydrogen production of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPA**	**CHPB**	**CHPD**	**CHPE**
0	0	0	0	0
24	29	28	29	64
48	125	119	119	109
72	177	169	168	140
96	205	202	198	162
120	225	221	219	177
144	236	232	232	188
168	244	240	241	198
192	248	242	247	204
216	248	243	251	209
240	248	243	251	213

Table I.16 Total hydrogen production of *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHSA**	**CHSB**	**CHSC**	**CHSD**	**CHSE**
0	0	0	0	0	0
24	26	30	38	43	104.8
48	103	108	128	145	176.8
72	155	159	181	203	228
96	189	194	201	243	254.4
120	214	220	226	261	276
144	230	236	229	267	284
168	237	243	232	271	290.4
192	242	248	233	273	292
216	244	251	234	273	296.8
240	244	251	234	273	296.8

Table I.17 Total hydrogen production of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPA**	**CWPF**	**CWPG**
0	0	0	0
24	13	38	40.5
48	84	87	83
72	123	122	118
96	173	138	130
120	208	154	141
144	235	194	154
168	242	210	165
192	246	216	174
216	248	230	182
240	248	238	189

Table I.18 Total hydrogen production of *R. capsulatus (DSM 1710)* + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWSB**	**CWSD**	**CWSF**
0	0	0	0
24	16	14	39
48	82	74	88
72	120	113	127
96	155	162	163
120	181	177	190
144	193	215	201
168	201	223	213
192	215	231	217
216	223	236	224
240	230	236	231

Table I.19 Total hydrogen production of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**PSA**	**PSB**	**PSC**	**PSD**	**PSE**	**PSF**	**PSH**
0	0	0	0	0	0	0	0
24	13	13	14	7	3	2	1
48	124	122	119	89	84	72	72
72	217	212	182	173	151	135	137
96	277	275	220	205	217	201	207
120	302	295	243	213	254	242	254
144	306	299	248	214	260	251	264
168	309	300	249	215	261	252	265
192	310	300	249	216	262	254	265
216	313	300	249	216	262	254	266
240	313	300	249	216	262	254	266

Table I.20 Total hydrogen production of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1H1**	**C2H1**	**C1H2**	**C2H2**	**CHA**	**CHB**	**CHC**
0	0	0	0	0	0	0	0
24	66	55.5	45	45	72	65.6	108
48	105	111	108	108	104.8	104	153.6
72	141	144	138	132	115.2	118.4	177.6
96	174	174	180	168	128	134.4	224
120	195	189	210	198	144	152	262.4
144	213	207	228	222	160	168	278.4
168	222	216	234	228	173.6	192	288
192	225	219	237	231	184	208	316.8
216	227	221	240	233	196	224	328
240	228	223	241	234	208	241.6	334.4

Table I.21 Total hydrogen production of *R. capsulatus (DSM 1710)* cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWA**	**CWB**	**CWD**
0	0	0	0
24	32	17	4.05
48	72	48	26.1
72	96.8	67	48.9
96	120	83	51.9
120	129.6	95	56.4
144	142.4	105	62.1
168	144	113	68.4
192	150.4	118	75.15
216	152	123	81.9
240	152	125	90.15

Table I.22 Total hydrogen production of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1H1**	**P2H1**	**Pal 3**	**PA**	**PB**	**PC**
0	0	0	0	0	0	0
24	6	6	0	2.5	3	4
48	12	12	30	13	9	11.2
72	72	72	102	70.5	73	107.2
96	147	150	156	140	140	184
120	204	207	201	180	188	253.6
144	228	228	219	218	220.5	300.8
168	231	231	225	238	241	315.2
192	234	237	234	243	248	317.6
216	244	246	242	245	249	323.2
240	245	248	243	245	249	327.2

Table I.23 Total hydrogen production of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S2H1**	**S1H2**	**Sph 3**	**SA**
0	0	0	0	0
24	6	3	0	4
48	12	15	12	4
72	72	72	87	57
96	96	129	150	124
120	120	168	189	172
144	174	183	210	212
168	183	192	222	226
192	195	192	228	229
216	204	202	232	231
240	212	210	234	232

Table I.24 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPSA**	**CHPSB**	**CHPSC**	**CHPSD**	**CHPSE**
0	0.6	0.6	0.6	0.6	0.6
24	2.47	2.99	3.1	2.82	2.23
48	2.77	3.2	3.18	3.1	3.4
72	3.12	3.52	3.2	3.24	3.52
96	3.23	3.91	3.29	3.98	3.26
120	3.19	2.94	2.73	2.64	2.81
144	2.63	2.5	2.67	2.59	2.75
168	2.68	2.38	2.95	3.15	2.76
192	2.55	2.34	2.73	2.8	2.69
216	2.48	2.18	2.41	2.46	2.37
240	2.54	2.22	2.59	2.66	2.51

Table I.25 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPSA**	**CWPSB**	**CWPSC**	**CWPSD**	**CWPSE**
0	0.6	0.6	0.6	0.6	0.6
24	2.73	2.68	2.64	2.73	2.46
48	2.77	2.97	2.73	2.74	3.23
72	3.16	3.12	2.13	3.18	3.32
96	3.26	3.24	3.45	3.34	3.36
120	3.4	3.46	3.15	3.47	3.4
144	3.14	2.95	3.04	2.65	2.66
168	2.98	2.87	3.21	2.7	2.75
192	3.06	3.14	3.06	2.71	2.64
216	2.6	2.55	2.47	2.45	2.53
240	2.55	2.62	2.72	2.45	2.43

Table I.26 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPA**	**CHPB**	**CHPC**	**CHPD**	**CHPE**	**CHPF**	**CHPG**
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	2.01	1.93	1.86	1.8	2.15	2.04	2.06
48	2.39	2.38	2.33	2.34	3.12	2.74	2.46
72	2.86	2.93	2.87	2.69	3.31	3.06	3.06
96	2.97	2.93	2.8	2.89	4.22	3.59	3.62
120	2.99	2.87	2.74	2.8	4.09	3.98	3.58
144	2.85	2.76	2.41	2.71	3.46	3.66	3.35
168	2.5	2.4	2.02	2.4	3.51	3.88	3.28
192	2.43	2.36	2.11	2.52	3.47	3.35	3.29
216	2.2	2.24	1.96	2.21	2.92	2.98	3.04
240	2.28	2.31	2.05	2.29	3.29	3.24	2.98

Table I.27 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHSA**	**CHSB**	**CHSC**	**CHSD**	**CHSE**	**CHSF**
0	0.4	0.4	0.4	0.4	0.4	0.4
24	2	2.01	1.95	2.02	2.07	2.13
48	2.52	2.52	2.32	2.42	2.25	2.74
72	2.89	2.8	2.69	2.73	2.82	3.06
96	3.08	2.96	2.71	2.83	2.93	3.87
120	3.11	2.83	2.53	2.92	3.01	3.73
144	3.03	2.82	2.41	2.64	3.07	3.47
168	2.91	2.64	2.33	2.5	2.92	3.37
192	2.65	2.48	2.08	2.29	2.77	3.46
216	2.38	2.47	2.15	2.32	2.45	3.02
240	2.32	2.45	2.17	2.34	2.53	3.14

Table I.28 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPA**	**CWPB**	**CWPC**	**CWPD**	**CWPE**	**CWPF**	**CWPG**	**CWPH**
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	1.53	1.54	1.57	1.57	2.18	1.95	2.04	1.82
48	2.09	2.12	2.13	2.13	2.26	2.52	2.57	2.01
72	2.3	2.33	2.36	2.33	2.62	2.85	3.26	2.53
96	2.53	2.53	2.51	2.55	2.74	4.7	3.74	3.6
120	2.78	2.77	2.82	2.78	2.86	3.58	3.34	2.94
144	2.69	2.62	2.59	2.48	2.9	3.1	3.06	2.67
168	2.5	2.27	2.37	2.15	2.8	3.16	3.26	2.87
192	2.31	1.99	2.08	1.94	2.49	3.11	3.13	3.01
216	2.36	2.06	2.08	2.04	2.41	2.64	2.92	2.73
240	2.41	2.11	2.1	2.07	2.45	2.64	2.89	2.91

Table I.29 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus (DSM 1710)* + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWSA**	**CWSB**	**CWSC**	**CWSD**	**CWSE**	**CWSF**
0	0.4	0.4	0.4	0.4	0.4	0.4
24	1.62	1.62	1.64	1.6	2.02	1.92
48	2.22	2.23	2.18	2.13	2.95	2.74
72	2.54	2.63	2.45	2.42	3.16	3.16
96	2.73	2.71	2.51	2.64	4.14	4.23
120	3.19	3.06	2.78	2.75	3.91	3.74
144	2.95	2.88	2.78	2.63	3.3	3.25
168	2.93	2.88	2.46	2.54	3.23	1.93
192	2.5	2.51	2.24	2.34	3.12	3.26
216	2.66	2.64	2.06	2.32	2.85	2.94
240	2.7	2.67	2.01	2.35	2.96	2.99

Table I.30 Absorbance values at 660nm measured with 1 / 10 dilution of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**PSA**	**PSB**	**PSC**	**PSD**	**PSE**	**PSF**	**PSG**	**PSH**
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	1.86	2.01	1.83	1.44	1.17	1.07	1.06	1.03
48	2.51	2.58	2.63	2.37	2.63	2.47	2.48	2.48
72	2.55	2.54	2.67	2.59	2.81	2.73	2.73	2.7
96	2.57	2.56	2.63	2.62	2.94	2.88	2.84	2.75
120	2.33	2.46	2.67	2.62	2.98	3.03	2.96	2.86
144	2.32	2.36	2.44	2.43	2.84	2.73	2.76	2.57
168	2.18	2.28	2.34	2.34	2.62	2.63	2.57	2.57
192	2.13	2.14	2.27	2.24	2.56	2.57	2.54	2.62
216	2.17	2.15	2.15	2.14	2.3	2.41	2.38	2.48
240	2.19	2.12	2.28	2.22	2.34	2.48	2.42	2.54

Table I.31 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1H1**	**C2H1**	**C1H2**	**C2H2**	**CHA**	**CHB**	**CHC**	**CHD**
0	0.21	0.21	0.17	0.18	0.2	0.2	0.2	0.2
24	1.47	1.46	1.66	1.74	1.84	1.59	1.47	1.99
48	1.74	1.82	2.07	2.17	1.92	2.06	1.88	2.45
72	1.82	1.92	2.21	2.22	2.38	2.48	2.56	2.63
96	2.1	2.3	2.21	2.22	2.63	2.96	2.67	2.89
120	2.17	2.26	2.33	2.31	2.25	2.39	2.72	2.33
144	2.24	2.29	2.4	2.43	2.3	2.43	2.22	2.74
168	1.32	2.21	2.38	2.37	2.3	2.5	2.31	2.4
192	1.29	2.18	2.3	2.32	2.25	2.27	2	2.48
216	2.17	2.11	2.28	2.26	1.92	1.96	1.48	2.2
240	2.05	2.07	2.18	2.2	2.05	2.07	1.54	2.24

Table I.32 Absorbance values at 660nm measured with 1 / 10 dilution of *R. capsulatus (DSM 1710)* cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWA**	**CWB**	**CWC**	**CWD**
0	0.2	0.2	0.2	0.2
24	1.27	1.6	1.28	1.24
48	1.69	2.05	1.54	1.61
72	2.16	2.17	2.19	1.82
96	2.66	2.31	2.31	2.06
120	2.02	2.11	2.11	1.57
144	2.05	2.09	1.83	1.7
168	1.98	1.79	2.02	1.62
192	1.96	1.89	1.93	1.48
216	1.71	1.6	1.47	1.41
240	1.92	1.8	1.32	1.54

Table I.33 Absorbance values at 660nm measured with 1 / 10 dilution of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1H1**	**P2H1**	**P1H2**	**P2H2**	**PA**	**PB**	**PC**	**PD**
0	0.24	0.23	0.23	0.23	0.2	0.2	0.2	0.2
24	0.34	0.33	0.34	0.33	0.57	0.62	0.75	0.65
48	0.87	0.88	0.74	0.83	1.89	1.94	1.51	0.57
72	1.97	1.97	1.84	1.86	2.63	2.73	2.25	1.45
96	2	2.11	1.94	1.97	2.35	2.76	2.31	3.02
120	2.52	2.19	2.14	2.17	2.99	2.95	2.44	2.92
144	2.11	2.27	2.23	2.26	2.6	2.87	2.2	3.08
168	2.23	2.35	2.31	2.35	2.81	2.72	2.18	3.1
192	2.25	2.37	2.33	2.38	2.55	2.6	2.2	3.09
216	2.27	2.4	2.35	2.41	2.68	2.47	2.11	3.05
240	2.24	2.41	2.36	2.4	2.51	2.52	2.18	2.86

Table I.34 Absorbance values at 660nm measured with 1 / 10 dilution of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1H1**	**S2H1**	**S1H2**	**S2H2**	**SA**	**SB**	**SC**	**SD**
0	0.22	0.22	0.26	0.26	0.2	0.2	0.2	0.2
24	0.3	0.32	0.39	0.37	0.46	0.49	0.62	0.41
48	0.61	0.67	0.82	0.78	1.47	1.57	0.99	0.47
72	1.67	2.03	2.25	2.23	2.56	2.53	2.04	1.73
96	2.23	2.31	2.43	2.57	3.05	3.53	2.36	2.4
120	2.3	2.4	2.72	2.8	3.16	3.36	2.62	2.9
144	2.33	2.41	2.79	2.86	3.15	3.46	2.66	2.86
168	2.42	2.49	2.83	3.01	3.16	3.29	2.61	3.26
192	2.54	2.51	2.84	3.04	2.95	3.1	2.48	3.19
216	2.6	2.55	2.85	2.98	2.68	2.59	2.26	2.92
240	2.59	2.53	2.84	2.96	2.79	2.38	2.13	3.13

Table I.35 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPSA**	**CHPSB**	**CHPSC**	**CHPSD**	**CHPSE**
0	0.6	0.6	0.6	0.6	0.6
24	2.314	2.432	2.588	2.438	2.358
48	2.596	2.72	2.732	2.668	2.7
72	2.77	2.864	2.812	2.738	2.814
96	2.88	3.296	2.77	2.92	2.564
120	2.64	2.61	2.664	2.708	2.604
144	2.446	2.354	2.556	2.598	2.438
168	2.482	2.102	2.464	2.494	2.362
192	2.404	2.62	2.358	2.402	2.296
216	2.396	2.044	2.366	2.416	2.292
240	2.31	2.066	2.416	2.456	2.318

Table I.36 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 ℃ under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPSA**	**CWPSB**	**CWPSC**	**CWPSD**	**CWPSE**
0	0.6	0.6	0.6	0.6	0.6
24	2.416	2.384	2.424	2.388	2.374
48	2.638	2.614	2.68	2.624	2.856
72	2.9	3.812	3.02	2.776	2.922
96	2.804	2.798	3.084	2.76	2.806
120	2.852	2.834	2.808	2.836	2.816
144	2.704	2.714	2.66	2.384	1.512
168	2.614	2.608	2.586	2.35	2.44
192	2.488	2.508	2.446	2.25	2.44
216	2.526	2.552	2.504	2.264	2.41
240	2.562	2.574	2.51	2.322	2.422

Table I.37 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPA**	**CHPB**	**CHPC**	**CHPD**	**CHPE**	**CHPF**	**CHPG**
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	1.9	1.904	1.912	1.842	2.204	2.066	2.052
48	2.24	2.282	2.256	2.24	2.894	2.576	3.032
72	2.57	2.632	2.63	2.622	2.972	2.362	2.87
96	2.684	2.682	2.656	2.67	2.87	2.886	2.87
120	2.668	2.688	2.572	2.638	2.958	2.97	2.924
144	2.53	2.51	2.368	2.598	2.968	3.004	2.946
168	2.352	2.37	2.168	2.516	2.93	2.848	2.754
192	2.202	2.196	2.04	2.362	2.82	2.76	2.664
216	2.026	2.088	1.966	2.184	2.772	2.712	2.592
240	2.006	2.058	1.936	2.104	2.72	2.68	2.534

Table I.38 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHSA**	**CHSB**	**CHSC**	**CHSD**	**CHSE**	**CHSF**
0	0.4	0.4	0.4	0.4	0.4	0.4
24	1.9	1.9	1.88	1.94	1.986	2.1
48	2.3	2.296	2.26	2.334	2.006	2.65
72	2.634	2.616	2.494	2.612	2.73	2.874
96	2.734	2.708	2.556	2.554	2.562	2.8
120	2.746	2.67	2.542	2.624	2.708	2.834
144	2.664	2.63	2.366	2.532	2.608	2.922
168	2.562	2.478	2.214	2.364	2.5	2.784
192	2.438	2.38	2.124	2.254	2.36	2.692
216	2.312	2.324	2.054	2.16	2.386	2.672
240	2.304	2.314	2.044	2.16	2.374	2.666

Table I.39 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPA**	**CWPB**	**CWPC**	**CWPD**	**CWPE**	**CWPF**	**CWPG**	**CWPH**
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	1.47	1.504	1.524	1.54	1.88	1.958	1.966	1.752
48	1.922	1.964	1.97	2.016	2.162	2.48	2.474	2.018
72	2.322	2.282	2.29	2.314	2.512	2.73	2.76	2.202
96	2.5	2.414	2.454	2.458	2.498	2.746	2.76	2.544
120	2.596	2.47	2.548	2.518	2.632	2.822	2.822	2.41
144	2.53	2.392	2.444	2.408	2.5	2.814	2.822	2.422
168	2.428	2.28	2.328	2.146	2.468	2.682	2.74	2.476
192	2.28	2	2.034	1.974	2.346	2.594	2.63	2.48
216	2.18	1.958	1.976	1.982	2.316	2.484	2.578	2.512
240	2.1	1.952	1.964	1.988	2.306	2.428	2.53	2.516

Table I.40 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus (DSM 1710)* + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWSA**	**CWSB**	**CWSC**	**CWSD**	**CWSE**	**CWSF**
0	0.4	0.4	0.4	0.4	0.4	0.4
24	1.588	1.58	1.55	1.532	1.96	1.94
48	2.144	2.108	2.08	2.036	2.608	2.532
72	2.432	2.44	2.392	2.366	3.036	3.066
96	2.624	2.628	2.54	2.508	2.844	2.724
120	2.756	2.706	2.572	2.564	2.812	2.92
144	2.76	2.754	2.47	2.594	2.824	2.864
168	2.736	2.698	2.35	2.456	2.724	2.754
192	2.608	2.574	2.1	2.27	2.702	2.714
216	2.484	2.454	2.042	2.15	2.676	2.68
240	2.446	2.426	2.024	2.138	2.644	2.64

Table I.41 Absorbance values at 660nm measured with 1 / 2 dilution of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**PSA**	**PSB**	**PSC**	**PSD**	**PSE**	**PSF**	**PSG**	**PSH**
0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	1.8	1.86	1.74	1.3	1.07	1.002	0.99	1.008
48	2.35	2.4	2.34	2.216	2.244	2.232	2.18	2.19
72	2.454	2.462	2.518	2.388	2.52	2.49	2.43	2.44
96	2.382	2.468	2.488	2.424	2.63	2.612	2.572	2.582
120	2.244	2.344	2.402	2.406	2.626	2.664	2.636	2.552
144	2.138	2.27	2.324	2.32	2.556	2.568	2.534	2.564
168	2.096	2.182	2.246	2.182	2.45	2.47	2.438	2.468
192	2.02	2.086	2.168	2.118	2.376	2.312	2.324	2.412
216	1.928	2.08	2.184	2.092	2.1	2.164	2.198	2.312
240	2.004	2.086	2.196	2.088	2.108	2.142	2.188	2.304

Table I.42 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1H1**	**C2H1**	**C1H2**	**C2H2**	**CHA**	**CHB**	**CHC**	**CHD**
0	0.21	0.21	0.194	0.2	0.2	0.2	0.2	0.2
24	1.466	1.488	1.584	1.63	1.676	1.526	1.402	1.702
48	1.716	1.82	1.92	1.97	1.894	1.922	1.914	1.856
72	1.784	1.858	2.026	2.036	2.144	2.026	2.026	1.86
96	2.104	2.026	2.124	2.206	1.984	2.254	1.948	1.952
120	2.044	2.064	2.206	2.214	2.052	2.102	2.206	2.22
144	2.096	2.038	2.258	2.228	2.044	2.122	2.102	2.262
168	2.164	2.106	2.264	2.208	2.012	2.092	1.992	2.25
192	2.182	2.122	2.226	2.196	1.884	2.05	1.748	2.16
216	2.204	2.108	2.204	2.188	1.952	2.01	1.608	2.206
240	2.188	2.098	2.198	2.136	1.924	1.93	1.56	2.134

Table I.43 Absorbance values at 660nm measured with 1 / 2 dilution of *R. capsulatus (DSM 1710)* cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWA**	**CWB**	**CWC**	**CWD**
0	0.2	0.2	0.2	0.2
24	1.266	1.424	1.234	1.25
48	1.576	1.656	1.58	1.386
72	1.996	2.07	2.098	1.424
96	1.72	1.768	1.736	1.532
120	1.826	1.882	2.074	1.47
144	1.868	1.782	1.82	1.488
168	1.79	1.81	1.79	1.512
192	1.71	1.684	1.638	1.44
216	1.734	1.71	1.564	1.42
240	1.7	1.692	1.47	1.42

Table I.44 Absorbance values at 660nm measured with 1 / 2 dilution of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1H1**	**P2H1**	**P1H2**	**P2H2**	**PA**	**PB**	**PC**	**PD**
0	0.208	0.212	0.204	0.218	0.2	0.2	0.2	0.2
24	0.342	0.336	0.304	0.276	0.536	0.516	0.576	0.646
48	0.806	0.894	0.734	0.8	1.512	1.484	1.36	0.614
72	1.836	1.86	1.696	1.782	2.274	2.278	2.054	1.194
96	1.908	1.936	1.828	1.894	2.694	2.418	1.972	2.152
120	1.964	1.972	1.946	1.926	2.432	2.464	2.234	2.394
144	1.988	1.984	2.068	2.162	2.406	2.424	2.044	2.476
168	2.028	1.968	2.082	2.224	1.412	2.396	2.048	2.646
192	2.048	1.978	2.122	2.302	2.292	2.444	1.952	2.624
216	2.104	1.98	2.104	2.236	2.302	2.268	1.984	2.578
240	2.146	2.046	2.088	2.184	2.236	2.276	1.976	2.502

Table I.45 Absorbance values at 660nm measured with 1 / 2 dilution of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1H1**	**S2H1**	**S1H2**	**S2H2**	**SA**	**SB**	**SC**	**SD**
0	0.2	0.212	0.23	0.22	0.2	0.2	0.2	0.2
24	0.284	0.304	0.36	0.354	0.468	0.464	0.572	0.342
48	0.556	0.638	0.826	0.756	1.19	1.304	0.994	0.512
72	1.494	1.854	2.058	2.06	2.286	2.28	1.932	1.322
96	2.028	2.174	2.262	2.354	2.288	2.534	2.1	1.918
120	2.116	2.218	2.39	2.46	2.454	2.696	2.444	2.304
144	2.204	2.306	2.472	2.542	2.488	2.786	2.282	2.41
168	2.194	2.302	2.516	2.588	2.512	2.7	2.23	2.514
192	2.176	2.286	2.532	2.526	2.508	2.614	2.098	2.5
216	2.164	2.298	2.508	2.462	2.472	2.418	2.006	2.598
240	2.172	2.288	2.492	2.476	2.42	2.18	2.01	2.678

Table I.46 pH values of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPSA**	**CHPSB**	**CHPSC**	**CHPSD**	**CHPSE**
0	6.4	6.4	6.4	6.4	6.4
24	6.793	7.218	7.305	7.303	7.225
48	7.181	7.224	7.287	7.329	7.333
72	6.955	7.081	7.359	7.053	7.171
96	7.104	7.1	7.227	7.14	7.152
120	6.973	6.954	7.253	7.126	7.215
144	7.047	6.982	7.105	7.082	7.076
168	7.116	7.122	7.116	7.168	7.181
192	7.043	6.966	7.129	7.178	7.166
216	7.136	7.071	7.116	7.141	7.172
240	7.125	7.2	7.168	7.171	7.172

Table I.47 pH values of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPSA**	**CWPSB**	**CWPSC**	**CWPSD**	**CWPSE**
0	6.4	6.4	6.4	6.4	6.4
24	7.306	7.275	7.277	7.26	7.282
48	7.278	7.227	7.355	7.31	7.274
72	7.087	7.171	7.113	7.252	7.207
96	7.199	7.281	7.328	7.259	7.117
120	7.139	7.147	6.945	7.282	7.241
144	7.215	7.154	6.987	7.135	7.272
168	7.26	7.237	7.234	7.262	7.251
192	7.182	7.234	7.237	7.307	7.216
216	7.278	7.215	7.222	7.301	7.428
240	7.268	7.253	7.234	7.286	7.278

Table I.48 pH values of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHPA**	**CHPB**	**CHPC**	**CHPD**	**CHPE**	**CHPF**	**CHPG**
0	6.4	6.4	6.4	6.4	6.4	6.4	6.4
24	6.95	7.132	7.143	7.202	7.261	7.179	7.163
48	7.225	7.278	7.235	7.222	7.166	7.235	7.307
72	7.161	7.278	7.235	7.327	7.313	7.362	7.288
96	7.295	7.335	7.279	7.387	7.24	7.221	7.293
120	6.993	7.361	6.956	7.056	7.445	7.417	7.349
144	7.19	7.239	7.159	7.29	7.391	7.477	7.405
168	7.192	7.169	7.159	7.382	7.552	7.447	7.354
192	6.903	7.109	7.033	7.226	7.298	7.321	7.323
216	6.939	6.96	7.339	7.231	7.423	7.443	7.384
240	6.946	6.812	7.452	7.252	7.351	7.353	7.331

Table I.49 pH values of *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CHSA**	**CHSB**	**CHSC**	**CHSD**	**CHSE**	**CHSF**
0	6.4	6.4	6.4	6.4	6.4	6.4
24	7.122	7.022	6.925	7.059	7.201	7.181
48	7.12	7.167	7.16	7.238	7.293	7.104
72	7.153	6.999	7.131	7.128	7.375	7.339
96	7.267	7.306	7.269	7.33	7.308	7.318
120	7.134	7.431	7.277	7.337	7.278	7.354
144	7.242	7.243	7.266	7.123	7.259	7.414
168	7.261	7.065	7.082	7.17	7.294	7.368
192	7.089	7.185	7.102	7.157	7.218	7.259
216	7.023	7.126	6.959	6.936	7.243	7.351
240	7.018	7.035	6.937	6.904	7.3	7.336

Table I.50 pH values of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWPA**	**CWPB**	**CWPC**	**CWPD**	**CWPE**	**CWPF**	**CWPG**	**CWPH**
0	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
24	6.82	6.905	6.904	6.999	7.237	7.211	7.19	7.167
48	7.129	7.127	7.09	7.033	7.183	7.188	7.109	7.083
72	7.191	7.128	7.105	7.363	7.244	7.469	7.383	7.033
96	7.268	7.222	7.137	7.216	7.05	7.221	7.131	7.102
120	7.259	7.038	7.138	7.122	7.334	7.306	7.297	7.156
144	7.127	7.095	7.148	7.252	7.235	7.344	7.44	7.153
168	7.16	7.042	7.136	7.051	7.258	7.355	7.386	7.307
192	7.09	6.999	7.197	7.293	7.168	7.302	7.301	7.202
216	7.165	7.295	7.268	7.224	7.265	7.246	7.308	7.351
240	7.205	7.315	7.275	7.216	7.243	7.176	7.345	7.395

Table I.51 pH values of *R. capsulatus (DSM 1710)* + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWSA**	**CWSB**	**CWSC**	**CWSD**	**CWSE**	**CWSF**
0	6.4	6.4	6.4	6.4	6.4	6.4
24	7.002	6.816	6.813	6.983	7.174	7.169
48	6.946	7.126	7.001	7.114	7.19	7.149
72	7.314	7.364	7.169	7.067	7.182	7.295
96	7.201	7.254	7.264	7.284	7.35	7.296
120	7.225	7.235	7.119	7.197	7.297	7.358
144	7.391	7.317	7.227	7.279	7.311	7.355
168	7.38	7.273	7.145	7.222	7.385	7.383
192	7.286	7.323	7.103	7.121	7.275	7.277
216	7.215	7.375	7.218	7.167	7.368	7.372
240	7.164	7.393	7.235	7.183	7.36	7.343

Table I.52 pH values of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**PSA**	**PSB**	**PSC**	**PSD**	**PSE**	**PSF**	**PSG**	**PSH**
0	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
24	6.861	7.097	6.965	6.863	6.609	6.725	6.62	6.701
48	7.132	7.154	7.108	7.156	7.052	7.018	7.068	7.103
72	7.201	7.278	7.195	7.116	6.99	7.051	7.098	7.379
96	7.241	7.375	7.302	7.291	7.103	7.133	7.153	7.161
120	7.055	6.998	7.153	7.194	7.005	6.932	6.967	7.018
144	7.125	7.179	7.118	7.196	7.137	7.16	7.253	7.226
168	7.053	7.067	7.109	7.124	6.872	6.916	7.182	6.985
192	7.092	7.161	7.12	7.175	7.189	7.287	7.138	7.176
216	7.203	7.127	7.362	7.19	7.196	7.243	7.354	7.122
240	7.248	7.136	7.383	7.197	7.204	7.221	7.403	7.117

Table I.53 pH values of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C1H1**	**C2H1**	**C1H2**	**C2H2**	**CHA**	**CHB**	**CHC**	**CHD**
0	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
24	6.867	6.901	6.3	6.978	7.092	7.11	6.941	7.102
48	6.899	6.948	6.915	6.987	7.006	7.003	7.061	7.041
72	6.881	6.92	6.951	6.976	7.177	7.19	7.053	7.125
96	6.919	6.937	7.307	6.979	7.064	7.257	7.131	7.338
120	7.221	7.149	7.148	6.957	7.088	7.137	7.072	7.205
144	6.749	6.883	7.092	7.156	7.198	7.251	7.182	7.33
168	7.098	6.863	6.737	6.836	7.287	7.247	7.149	7.251
192	6.653	6.808	6.894	6.934	7.215	7.148	7.078	7.238
216	7.143	7.121	7.032	7.143	7.229	7.223	7.065	7.199
240	7.242	7.013	7.142	7.214	7.217	7.257	7.209	7.144

Table I.54 pH values of *R. capsulatus (DSM 1710)* cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CWA**	**CWB**	**CWC**	**CWD**
0	6.4	6.4	6.4	6.4
24	7.042	7.037	6.94	6.994
48	7.125	7.13	7.064	6.944
72	7.154	7.331	6.973	7.019
96	7.208	7.293	7.109	7.135
120	7.208	7.216	7.071	7.077
144	7.281	7.377	7.145	7.201
168	7.329	7.321	7.229	7.234
192	7.351	7.245	7.182	7.159
216	7.357	7.287	7.123	7.074
240	7.417	7.338	7.134	7.171

Table I.55 pH values of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P1H1**	**P2H1**	**Pal 3**	**PA**	**PB**	**PC**	**PD**
0	6.4	6.4	6.4	6.4	6.4	6.4	6.4
24	6.577	6.35	6.618	6.585	6.599	6.6	6.512
48	6.648	6.683	6.96	6.962	6.964	6.901	6.555
72	7.208	7.029	6.973	7.194	7.151	6.895	6.838
96	7.008	6.923	7.135	7.15	7.147	7.094	7.252
120	6.988	7.084	7.219	7.154	7.161	7.12	7.035
144	6.832	7.206	7.212	7.175	7.275	7.201	7.214
168	7.084	7.298	7.132	7.231	7.265	7.167	7.271
192	6.897	6.816	7.214	7.186	7.156	7.118	7.295
216	6.942	6.934	7.145	7.304	7.262	7.21	7.288
240	7.095	7.012	7.205	7.231	7.197	7.163	7.293

Table I.56 pH values of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S1H1**	**S2H1**	**S1H2**	**S2H2**	**SA**	**SB**	**SC**	**SD**
0	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
24	6.479	6.398	6.495	6.509	6.515	6.495	6.535	6.513
48	6.782	6.553	6.717	6.805	6.921	6.904	6.703	6.468
72	6.817	6.964	7.145	7.05	7.089	7.079	6.969	6.725
96	6.999	7.063	7.244	7.444	7.146	7.145	7.159	7.258
120	6.934	6.965	7.091	7.244	7.189	7.168	7.15	7.144
144	7.241	7.35	7.702	7.96	7.271	7.265	7.309	7.252
168	7.213	6.968	7.036	7.074	7.274	7.287	7.193	7.263
192	7.224	7.023	7.165	7.201	7.24	7.21	7.159	7.265
216	7.197	7.146	7.329	7.193	7.309	7.268	7.201	7.343
240	7.253	7.234	7.197	7.207	7.223	7.202	7.259	7.351

Table I.57 Acteate concentrations of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	CHPSA1a__2	CHPSA1b__3	CHPSB1a__4	CHPSB1b__5	CHPSC1a__6	CHPSC1b__8	CHPSD1a__9	CHPSD1b__10
0	40	40	40	40	40	40	40	40
24	13.7845474	13.7538936	13.9347364	13.9342655	14.4527366	14.0236246	13.8356246	14.2354725
48	6.84767664	6.34582968	6.18051312	6.38287267	6.49471889	6.33187635	6.49119647	6.81526645
72	2.41730468	1.73323389	2.5897086	2.61573273				
96	0.76962015		0.76830965	0.72548804	0.82966604	1.06970436	0.92980981	0.88097936
120								
144	0.57456931	0.57770036		0.54283032		0.63259986	0.51539474	
168								
192	0.47906544	0.49837589			0.45652122			
216								
240	0.38683605	0.34567382	0.30923295		0.35244594	0.26134641		

Table I.58 Acteate concentrations of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	CWPSA1a__11	CWPSA1b__12	CWPSB1a__14	CWPSB1b__15	CWPSC1a__16	CWPSC1b__17	CWPSD1a__18	CWPSD1b__20
0	40	40	40	40	40	40	40	40
24	15.8634597	15.1239582	16.0175682	15.2387344	15.7230986	14.8234825	16.3498946	15.2379485
48	8.14524934	8.29918931	7.95092282	7.84610728	7.72882601	8.11556111	7.94109581	7.54400064
72	3.22386335	3.17262617	3.35826441	3.21967682				
96	1.276022	1.38453352	1.21669866	1.1137373	1.03084083	0.98626067	0.90708319	0.86274919
120								
144	0.42677101	0.81625285	0.75646021	0.7617713	0.60292933	0.55573879	0.50620881	0.48644322
168								
192	0.25048871	0.24045804	0.46253537	0.33555991	0.37783607	0.30342245	0.3448946	0.26709847
216								
240	0.1138899		0.20677277	0.05691307	0.06091011	0.09512312	0.06576606	

Table I.59 Acteate concentrations of *R. capsulatus* hup- (YO3) + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	CPA1_CPA1_2	CPB1_CPB1_3	CPC1_CPC1_4	CPD1_CPD1_5
0	40	40	40	40
24	16.0203152	21.9550911	22.5680865	22.1722735
48	13.2576838	13.3894479	13.5693412	13.6182921
72	5.88155169	5.79748285	6.03413334	6.46637836
96	2.23360564	2.20178519	2.31484274	2.76392773
120	0.46390255	0.39159282	0.20466888	0.81126231
144	0.19878578	0.20299356	0.18839383	0.1971813
168		0.16268493	0.1336732	0.15738447
192		0.10652628	0.10841589	0.08462492
216		0.07818929	0.07871703	
240				

Table I.60 Acteate concentrations of *R. capsulatus* hup- (YO3) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	CSA1_CSA1_6	CSB1_CSB1_8	CSC1_CSC1_9	CSD1_CSD1_10
0	40	40	40	40
24	21.47992	21.1430106	21.7915597	21.3444103
48	13.5314641	14.059252	12.3366119	12.0531215
72	6.86080647	6.95677965	5.45833321	4.71160792
96	2.89581404	2.93766163	1.93649132	1.49804797
120	1.04023219	0.81414719	0.33488872	0.10830255
144	0.06741659		0.1805981	0.09780966
168		0.0419202		0.16217844
192				0.05301875
216	0.03338598		0.05669525	
240				

Table I.61 Acteate concentrations of *R. capsulatus (DSM 1710)* + *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	CWPA1_CWPA1_55	CWPB1_CWPB1_56	CWPC1_CWPC1_58	CWPD1_CWPD1_59
0	40	40	40	40
24	25.4773704	25.2481916	24.9104304	24.930157
48	16.2231777	16.1821944	16.1492334	15.5852562
72	10.3934069	10.357515	10.3020701	9.58532344
96	5.09251305	4.89941734	4.79657641	4.11078071
120	0.93924367	1.202802	0.84201311	
144			0.66819776	0.62418784
168				
192				
216				
240				

Table I.62 Acteate concentrations of *R. capsulatus (DSM 1710)* + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	CWSA1_CWSA1_50	CWSB1_CWSB1_52	CWSC1_CWSC1_53	CWSD1_CWSD1_54
0	40	40	40	40
24	24.8103273	25.8555996	25.250129	25.935149
48	17.3315685	17.0185068	15.8164114	16.9778546
72	11.6970646	10.9399438	9.76288614	11.0520504
96	6.82661825	6.4407704	4.35193473	5.61296563
120	2.84838087	2.5293156	0.45081167	1.33578276
144	0.97925655	0.93207132		
168			0.21863283	0.47027443
192				
216				
240				

Table I.63 Acteate concentrations of *R. palustris* (DSM 127) + *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	PSA1_PSA1_11	PSB1_PSB1_12	PSC1_PSC1_14	PSD1_PSD1_15	PSE1_PSE1_46	PSF1_PSF1_47	PSG1_PSG1_48	PSH1_PSH1_49
0	32	32	32	32	32	32	32	32
24	25.36382	24.91739	26.14155	28.28272	29.37471	30.24702	29.12997	30.45038
48	12.95609	11.07162	13.31772	14.65832	16.92465	17.02735	17.38967	18.13159
72	4.786928	4.680962	5.543288	6.490701	9.607602	10.56372	10.70469	10.2768
96	0.798205	0.528432	1.086472	1.726924	3.497321	4.217612	4.413927	4.566402
120	0.122304	0.118186	0.118953	0.198844	0.164686	0.213779	0.278519	0.288667
144	0.094567	0.085394	0.136719	0.041679	0.049879			
168			0.027427		0.047038			
192								
216								
240								

Table I.64 Acteate concentrations of *R. capsulatus* hup- (YO3) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**C-1-1**	**C-2-1**	**C-1-2**	**C-2-2**
0	40	40	40	40
24	20.2193283	22.9042946	24.1186184	23.0266975
48	16.3914323	15.0836749	14.9364468	13.9037996
72	9.60312328	8.70393499	7.4822086	7.58056194
96	6.06242783	3.6480647	3.42242598	3.68177654
120	0.89623257	1.24400851	1.1453665	1.39464919
144	0.16269733	0.11887336	0.24886652	0.16520145
168			0.15790513	0.13375112
192				
216				
240				

Table I.65 Acteate concentrations of *R. capsulatus (DSM 1710)* cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**CW-1-1**	**CW-1-2**	**CW-2-1**	**CW-2-2**
0	40	40	40	40
24	25.7345356	28.4987346	28.1983746	27.0432885
48	18.9346424	17.6298839	18.7243989	17.6239849
72	13.6983459	12.745985	12.2895985	12.3762374
96	8.49834988	6.03487474	6.94938393	7.17479398
120	1.39845889	1.7309499	2.39848983	2.50984309
144	0.59384989	0.53948748	1.44098499	0.93498784
168	0.25093489			0.38457988
192				0.10985839
216				
240				

Table I.66 Acteate concentrations of *R. palustris* (DSM 127) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**P-1-1**	**P-2-1**	**P-1-2**	**P-2-2**
0	40	40	40	40
24	33.5378461	33.0958236	32.7831232	31.5211873
48	25.4598761	26.4711163	28.0152651	25.980952
72	16.4656228	15.8203287	26.047036	24.944213
96	7.45484208	7.46409708	14.312073	13.0874423
120	1.26653147	1.22968859	6.14476823	4.8270474
144	0.12682847	0.12380546	0.70916341	0.36033553
168		0.06135993	0.06528613	
192				
216				
240				

Table I.67 Acteate concentrations of *R. sphaeroides* O.U.001 (DSM 5864) cultured on growth medium at 30 °C under anaerobic conditions and continuous illumination at 2000 lux.

Time (h)	**S-1-1**	**S-2-1**	**S-1-2**	**S-2-2**
0	40	40	40	40
24	29.4728232	30.2274735	31.9953277	30.6729298
48	24.3932744	22.6617165	25.7922734	22.0944045
72	21.3944114	18.8619736	15.121938	14.6914002
96	10.5319059	9.25015137	7.49779829	7.80097281
120	3.94667548	3.03797364	2.30380971	2.68227978
144	0.06230208	0.24246276	0.23261449	0.05089007
168		0.09678605	0.10248497	
192			0.0286965	
216				
240				