BIOLOGICAL HYDROGEN PRODUCTION ON ACETATE IN CONTINUOUS PANEL PHOTOBIOREACTORS USING RHODOBACTER CAPSULATUS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

DOMINIC DEO ANDROGA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMICAL ENGINEERING

JULY 2009

Approval of the thesis:

BIOLOGICAL HYDROGEN PRODUCTION ON ACETATE IN CONTINUOUS PANEL PHOTOBIOREACTORS USING RHODOBACTER CAPSULATUS

submitted by **DOMINIC DEO ANDROGA** in partial fulfillment of the requirements for the degree of **Master of Science in Chemical Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Gürkan Karakaş _____
Head of Department, **Chemical Engineering**

Prof. Dr. İnci Eroğlu _____
Supervisor, **Chemical Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Hayrettin Yücel _____
Chemical Engineering Dept., METU

Prof. Dr. İnci Eroğlu _____
Supervisor, Chemical Engineering Dept., METU

Prof. Dr. Ufuk Bakır _____
Chemical Engineering Dept., METU

Prof.Dr. Meral Yücel _____
Biology Department, METU

Dr. Gökhan Kars _____
Biology Department, Selçuk University

Date: 31st July, 2009

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Dominic Deo ANDROGA

Signature:

ABSTRACT

BIOLOGICAL HYDROGEN PRODUCTION ON ACETATE IN CONTINUOUS PANEL PHOTOBIOREACTORS USING RHODOBACTER CAPSULATUS

Androga, Dominic Deo

M.Sc., Department of Chemical Engineering

Supervisor: Prof. Dr. İnci Eroğlu

July 2009, 189 pages

Photobiological hydrogen production from organic acids occurs in the presence of light and under anaerobic conditions. Stable and optimized operation of the photobioreactors is the most challenging task in the photofermentation process. The main aim of this study was to achieve in long term, a stable and high hydrogen production on acetate, using the photosynthetic bacteria *Rhodobacter capsulatus* in continuous panel photobioreactors.

Rhodobacter capsulatus (DSM 1710), heat adapted *Rhodobacter capsulatus* (DSM 1710) and *Rhodobacter capsulatus* YO3 (Hup^-), a mutant strain, were tested in outdoor conditions, under natural sunlight in Ankara, Turkey. The experiments were carried out between September-December, 2008. Defined culture medium containing acetate (40 mM) and glutamate (2 mM) and a dilution rate of 0.8 l/ day were used. Steady hydrogen production (0.4 mmol $H_2/l_c.h$) was obtained using the *Rhodobacter capsulatus* YO3 (Hup^-)

mutant strain that was continuously operated for 69 days, but the cell concentration could not be kept at a steady value.

Further efforts were focused on achieving stable biomass concentration besides stable hydrogen production in continuous operation. The feed composition was optimized in indoor experiments, under controlled temperature and continuous illumination. Stable biomass (0.40 gdcw/l_c) and the highest hydrogen productivity (0.8 mmol H_2/l_c.h) were achieved using the feed media containing 40 mM acetate and 4 mM glutamate with a 10% (v/v) feed rate.

Moreover, the EU project HYVOLUTION aims to combine dark fermentation and photo fermentation process for the conversion of biomass to hydrogen. Effluents from the dark fementation contain high amount of ammonium, which inhibits phototrophic hydrogen production. After treatment it has been concluded that Gördes clinoptilolite zeolite effectively removes ammonium ion from the dark fermenter effluent of molasses.

Keywords: *R. capsulatus*, hydrogen, acetate, photobioreactor, clinoptilolite

ÖZ

SÜREKLI PANEL FOTOBİYOREAKTÖRLERDE FOTOSENTETİK RHODOBACTER CAPSULATUS BAKTERİSİ KULLANARAK ASETATTAN HİDROJEN ÜRETİMİ

Dominic Deo Androga
Yüksek Lisans, Kimya Mühendisliği Bölümü
Tez Yöneticisi: Prof. Dr. İnci Eroğlu

Temmuz 2009, 189 sayfa

Işık varlığında ve oksijensiz şartlarda organik asitten fotobiyolojik hidrojen üretimi gerçekleşir. Fotobiyoreaktörlerde sabit ve optime üretim fotofermantasyon süreçlerindeki en zorlayıcı görevdir. Bu çalışmanın asıl amacı sürekli panel fotobiyoreaktörlerde fotosentetik *Rhodobacter capsulatus* bakterisi kullanarak asetattan uzun vadeli, sabit ve yüksek hidrojen üretimini elde etmektir.

Türkiye Ankara'da, güneş ışığı altında açık havada *Rhodobacter capsulatus* (DSM 1710), ısıl uyarlanmış *Rhodobacter capsulatus* (DSM 1710) ve mutant *Rhodobacter capsulatus* YO3 (Hup^-) test edildi. Bu deneyler Eylül-Aralık 2008 tarihleri arasında yapıldı. Seyreltme hızı 0.8 l/gün olan ve asetat (40 mM) ve glutamat (2 mM) içeren tanımlanmış kültür ortamı kullanıldı. 69 gün boyunca sürekli çalıştırılan panel fotobiyoreaktörde mutant *Rhodobacter capsulatus* (Hup^-) kullanıldığında 0.4 mmol $H_2/l_c.h$ sabit hidrojen üretimi elde edildi fakat düzgün olmayan biyokütle büyümesi gözlendi.

Daha fazla çaba sürekli üretimde sabit hidrojen üretiminin yanısıra sabit biyokütle konsantrasyonunu elde etmek için odaklandı. Bu amaca ulaşmak için, sıcaklığı control edilen ve sürekli aydınlatılan kapalı alan deneylerinde besleme bileşimi optimize edildi. Sabit biyokütle (0.40 gdcw/ l_c) ve en yüksek hidrojen üretimi (0.8 mmol H_2/l_c.h) 40 mM asetat ve 4 mM glutamat içeren besleme ortamı kullanıldığında elde edildi.

Ayrıca EU HYVOLUTION projesinin asıl amacı biyokütlenin hidrojene çevrimi için ışıklı ve ışıksız fermantasyon süreçlerinin birleştirilmesidir. Işıksız fermantasyonun çıkış sıvısında yüksek miktarda bulunan amonyum fototrofik hidrojen üretimini engeller. Yapılan amonyum iyonlarını azaltma deneylerınden sonra Gördes klinoptilolit zeolitinin melas ışıksız fermentor çıkış sıvısındaki amonyum iyonlarını etkili bir şekilde ayırdığı sonucuna varıldı.

Anahtar Kelimeler: *R. capsulatus*, hidrojen, asetat, fotobioreaktör, klinoptilolit

To My Family

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my supervisor, Prof. Dr. İnci Eroğlu for her endless guidance, advice and support throughout this research. I would also like to thank Prof. Dr. Meral Yücel and Prof. Dr. Ufuk Gündüz for their contribution to this study through their ideas and suggestions.

I also thank Dr. Ebru Özgür, Dr. Başar Uyar, Dr. Yavuz Öztürk and Dr. Gökhan Kars for their support and considerable contribution to my study through their suggestions and recommendations.

I am grateful to my labmates S.Gökçe Avcıoğlu, Nilüfer Afşar, Kamal Elkahlout, Pelin Sevinç, Muazzez Gürgan, Begüm Peksel, Efe Boran, Endam Özkan and Emrah Sağır for their friendship, collaboration and unending assistance. I greatly appreciate the discussions, suggestions and help you gave in carrying out some of the procedures.

I acknowledge Gülten Orakçı for her help with the HPLC analyses and Kerime Güney for the clinoptilolite zeolite chemical analysis at the Chemical Engineering Department. I also thank Prof. Dr. Hayrettin Yücel for giving samples of Gördes clinoptilolite zeolite for the ammonium ion reduction study. I greatly appreciate Ahmad Bahaalddin's help with the clinoptilolite zeolite experiments too.

Lastly, I sincerely thank my family for their invaluable support and love. I especially thank my parents for their love, encouragement and trust in me. I am grateful to my friends Mustafa Yalın, Deniz Barer and Bahram Lotfi Sadigh for their help and moral support.

This research study was supported by METU with project BAP No: 2006-07-020001, BAP No: 07-02-2009-0001 and the EU 6th Framework Integrated Project 019825 (HYVOLUTION).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

A: Irradiated area (m^2)

Ac: Acetate

Acetyl-CoA: Acetyl Coenzyme A

ATP: Adenosine tri-Phosphate

bchla: Bacterirochlorophyll

BR : Biomass Recycle

C/N: Carbon to Nitrogen Ratio

CPBR: Continuous Panel Photobioreactor

FAB: Fast Atom Bombardment

FID: Flame Ionization Detector

GC: Gas Chromatography

gdcw: Gram Dry Cell Weight of Bacteria

Glu: Glutamate

H_2: Hydrogen Gas

Hup^- : Uptake Hydrogenase Deficient (mutant)

HPLC: High Performance Liquid Chromatography

HRT: Hydraulic Retention Time

i.d: Internal Diameter

I: Light intensity (W/m^2)

l: Litre

k_c: Apparent Specific Growth Rate (h^{-1})

Lux: Luxmeter

mM: Millimolar

Me-3HB: Methyl 3-Hydroxybutyric Acid

PHB: Polyhydroxybutyrate

PNS: Purple Non-Sulphur

t: time (hours)

V: volume (ml or l)

W: Watt

X: Cell Concentration (gdcw/l_c)

X_{max}: Maximum Cell Concentration

X_o : Initial Cell Concentration

Greek Letters:

η: Light conversion efficiency (%)

μ: Specific growth rate constant (h^{-1})

ρ: Density (g/l)

μl: Microlitre

CHAPTER 1

INTRODUCTION

The 20th century saw the biggest increase in the world population in human history. The green revolution led to massive increases in agricultural productivity, technological and medical advances; mortality rate decreased in many countries worldwide. The current world population, estimated at 6.7 billion, is projected to reach 7 billion in early 2012 and surpass 9 billion by 2050 (UN Population Division/DESA, 2009). This will lead to more demand on energy. Energy is of paramount importance for a modern industrialized economy; it is critical for economic growth and progress. Currently, the energy consumed mainly comes from fossil fuels (oil, coal and gas) and nuclear sources. However, the fossil fuel reserves are limited and a decline in production is projected in the future. Also, fossil fuels lead to the emission of greenhouse gases (CO_x, NO_x and SO_x) that are harmful to mankind and the environment. For this reason, much emphasis is being laid on developing alternative energy sources that are reliable, renewable, cheap and environmentally friendly. The use of hydrogen as a future energy resource is proposed.

Hydrogen is the lightest and most plentiful element in the universe. It is a rich energy carrier that has the highest energy content compared to other hydrocarbon fuels. On combustion, it produces water as the main product and so is considered a ‘‘clean’’ fuel. It is used in a variety of fields; the petroleum industry (hydrocracking), the chemical industry (production of ammonia), the food industry (hydrogenation of fats), as a shielding gas in welding, as a coolant gas, reactant in nuclear fission and as a transportation fuel (fuel cells).

Currently, hydrogen is mainly produced from fossil fuels through processes like natural gas steam reformation, coal gasification and the

electrolytic splitting of water. It can also be produced from biomass using thermochemical processes like biomass pyrolysis and gasification and biologically, using microorganisms. Among the stated hydrogen production processes, biological hydrogen production is the least energy intensive and the most environmental benign process. Moreover, it is renewable and facilitates the utilization of the available natural resources; sunlight and agricultural wastes.

Biologically, hydrogen can be produced through processes such as the biophotolysis of water using algae and cyanobacteria, fermentation of organic compounds using fermentative bacteria, photo decomposition of organic compounds using photosynthetic bacteria and a combination of the above processes. Microalgae and cyanobacteria are able to biologically split water into hydrogen and oxygen using sunlight energy and carbondioxide from the atmosphere. Under anerobic conditions and in the absence of light, some bacteria can break down organic matter (agricultural wastes like molasses) to produce hydrogen and lower molecular weight organic acids. Photosynthetic bacteria can convert organic acids into hydrogen and carbondioxide using sunlight energy and nitrogenase enzyme, under anaerobic conditions. To avoid competition with food production, biological hydrogen production should be dependent on disposed biomass in which main sources are sugars and their polymers (Asada *et al*., 2008).

Hydrogen production by the photo decomposition of organic compounds using photosynthetic bacteria is considered the most favourable method for biological hydrogen production. This is because of the high conversion efficiency and versatility in using different substrates.

The efficiency of biological hydrogen production processes can be increased by integration of the different processes. An example is the combination of the dark fermentation and photofermentation systems to achieve maximum conversion of substrates and high hydrogen yield. The lower molecular weight organic acids produced in the dark fermentation process can be used as feedstock to photosynthetic bacteria in photofermentation systems.

This would lead to the nearly all, if not complete utilization of the initial substrate for hydrogen production. Such systems would enable hydrogen production at an industrial scale be feasible and cost effective.

‘‘HYVOLUTION’’, an acronym for “Non-thermal production of pure hydrogen from biomass”, is a European Union 6th framework integrated project with a concept of generating hydrogen using combined thermophilic dark fermentatative and phototrophic bacteria. The main objective of this project is to develop a two-stage bioprocess for the cost-effective production of pure hydrogen at small scale from locally obtained biomass feedstock. The bioprocess starts with a thermophilic fermentation of feedstock to hydrogen, carbondioxide and intermediates. Then all the intermediates produced are converted to more hydrogen and carbondioxide in consecutive photoheterotrophic fermentation. 75% efficiency (nearly 9 mol of hydrogen per mole of hexose) is achieved.

A major target of the HYVOLUTION project is to form a basis for the construction of a plant that will enable the conversion of biomass to pure hydrogen. The project is divided into seven work packages in which each partner is assigned a task; biomass, thermophilic fermentation, photo fermentation, gas upgrading, system integration, societal integration and training. These work packages represent modules which will eventually be combined to form a blue print for a hydrogen production plant. Major scientific and technological objectives include the development of bioreactor prototypes for thermophilic and photofermentation and the design of an optimized industrial scale hydrogen production plant that accounts for proper material usage and up-to date control and monitoring equipment.

Besides scientific and technological objectives, also socio-economic activities are included to increase public awareness and societal acceptance, and for identification of future opportunities, stakeholders and legal consequences of this specific bioprocess for decentral hydrogen production (Classen and Vrije, 2006).

The project that was started in 2006, involves 11 EU countries, Turkey, Russia and South Africa. METU Biohydrogen Group leads the photofermentation research of this five year project. The group has been studying hydrogen production by photosynthetic bacteria with respect to kinetics, biochemistry and process development and performed the design, construction and operation of laboratory scale reactors and an outdoor pilot scale photo bioreactor for hydrogen production.

Hydrogen production by photosynthetic bacteria occurs in the presence of light under anerobic and nitrogen limited conditions (high C/N ratio). The mechanism involves three main components; enzyme systems (nitrogenase and hydrogenase), carbon flow (TCA) cycle and the photosynthetic membrane unit. Organic substrates, such as acetate and lactate, are broken down in the TCA cycle to reducing equivalents (electrons and protons) and carbondioxide. The photosynthetic centre of the bacteria captures light and converts it into energy (ATP). Then the nitrogenase enzyme, under nitrogen limitation and excess electrons, enzyme utilizes the ATP to convert the electrons from the TCA cycle into hydrogen. Hydrogenase enzyme is an antagonistic enzyme which can either convert hydrogen back to electrons or form hydrogen from electrons, depending on the redox status of the culture.

By-products of photofermentative hydrogen production include polyhydroxybutyrate (PHB), carotenoid and biomass. PHB is a biodegradable thermoplastic that is a suitable substitute for petrochemical plastics. Carotenoid is a pigment that can be used as a food colorant and antioxidant. Biomass produced can be used as livestock feed supplement because it contains cells rich in protein.

An important factor in photofermentative hydrogen production is the successful design and operation of the photobioreactor. An elaborate simple design with low material and production costs and high utilization of sunlight within the optimum wavelength is desired. Flat panel type photobioreactors have the advantage of being stacked together to provide a large illuminated surface in a small ground area and can be oriented for optimal exposure.

Different organic substrates and photosynthetic bacterial strains have been tested for hydrogen production by the METU Biohydrogen Research Group. Comparisons of the different PNS bacteria that the group worked on for hydrogen productivity showed that the *Rhodobacter capsulatus* performed well in acetate containing media. Most of the experiments carried out were in batch mode and so different strains of this bacteria remain to be tested in continuous systems for hydrogen production in long term (Türkaslan *et al*., 1998; Eroğlu *et al*., 1999; Yiğit *et al*., 2000; Suludere, 2001; Koku *et al*., 2002; Koku *et al*., 2003; Eroğlu *et al*., 2004, Eroğlu *et al*., 2006, Öztürk *et al*., 2006; Uyar *et al*., 2007; Eroğlu *et al*., 2008; Özgür *et al*., 2009). Acetate is a central intermediate in the overall carbon cycle and can be easily utilised by the photosynthetic bacteria. Besides it has a reasonable cost and is a major product of the dark fermentation process. So research in the photofermentation process using acetate gives an opportunity to integrate the dark fermentation and photofermentation processes to achieve optimal hydrogen production.

An obstacle in the utilization of the dark ferment effluent (DFE) as feed to the photofermentation process is the presence of ammonium ion in the effluent. Ammonia inhibits nitrogenase enzyme, cutting down the hydrogen production capability of the PNS bacteria. A possible means of reducing the ammonia content in the DFE is the use of natural zeolites such as clinoptilolite. It is aimed to search the applicability of Gördes clinoptilolite to remove ammonium ions from the real DFE such as molasses for process integration of WP2 Thermophilic fermentation with WP3 Photofermentation steps in the HYVOLUTION Project.

The main objective of this study is to achieve a stable and high hydrogen production on acetate using *Rhodobacter capsulatus* in continuous panel photobioreactors in long term in outdoor conditions.

CHAPTER 2

LITERATURE SURVEY

2.1 Hydrogen

2.1.1 Properties and Uses of Hydrogen

Hydrogen, the first element on the chemical periodic table, is a very light, odourless and colourless gas. It is the most abundant element in the universe, making up about three quarters of all matter (Das and Veziroğlu, 2001). It has a 0.09 g/l density, -253 ^{0}C boiling point and is highly diffusive in air with a diffusion coefficient of 0.61 cm^3/s. Gaseous hydrogen burns in air at volume concentrations between 4 - 74.5% and has the highest energy to weight ratio of all fuels. 1 kg of hydrogen contains the same amount of energy as 2.1 kg of natural gas or 2.8 kg of gasoline (BACAS report, 2006). On combustion, hydrogen produces water as the main product, thus considered a clean non-polluting fuel. Shown in Equation (1) is the hydrogen combustion reaction. Also, compared to other gaseous fuels like water gas, it is harmless to humans and the environment (Bockris *et al*., 1981; Suzuki *et al*., 1982).

$$2\ H_2\ (g) + O_2\ (g) \rightarrow 2\ H_2O\ (l) + 286\ kJ/mol \qquad (1)$$

The unique physical properties and environmental benignity make hydrogen a major future energy carrier. Currently, it is mainly used in the petroleum industry (hydrocracking and hydrodesulphurization), the chemical industry (production of ammonia and methanol) and food industry (hydrogenation of fats). Other uses of hydrogen are as a shielding gas in welding, a coolant gas in electrical generators, reactant in nuclear fission and as fuel in rocket engines and fuel cells (Czuppon *et al*., 1994; Veziroğlu *et al*., 1995; Ramachandran *et al*., 1998).

2.1.2 Hydrogen Production Processes

Hydrogen is mostly produced from fossil fuels using processes like steam reformation of natural gas, partial oxidation and coal gasification. Electrolysis of water, pyrolysis of biomass and biological production are the other production methods employed (Hassmann *et al*., 1993).

Steam reformation of natural gas and other lighter hydrocarbons like naphtha account for nearly 80-85% of hydrogen produced today (Simpson *et al*., 2007). In this process, natural gas and steam are passed over nickel-based catalyst and reacted in a reactor at temperatures between 650 -700 ^{0}C. The products are a mixture of hydrogen and carbonmonoxide. Steam reformation is currently the least expensive method of hydrogen production but the technology is suitable for large reformers (100,000 tons per year) where yields higher than 80% can be achieved. Smaller scale reformers show lower efficiencies (Mc. Auliffe *et al*., 1980; BACAS Report, 2006).

Coal gasification is another industrially applied method for hydrogen production. It involves the reaction of coal with oxygen and steam in a high-temperature and high-pressure reactor at temperatures exceeding 700 ^{0}C. Syngas consisting of hydrogen and carbonmonoxide mixed with steam and cardondioxide is produced. Hydrogen production from coal gasification is highly practical with the available coal reserves worldwide, but emission of carbondioxide is a major environmental concern. Technologies like oxygen-blown gasification with carbondioxide capture and sequestration are applicable but still costly compared to other industrial processes like steam reformation (Collot, 2002).

Electrolytic splitting of water into hydrogen and oxygen is another widely used method for hydrogen production. It is mostly used in small scale applications when high purity of hydrogen is required. The application of the electrolytic process offers the opportunity to utilize renewable sources like solar, wind and hydropower but requires lots of electricity and is restricted to the regions with the available resources, for example hydroelectricity.

The aforementioned industrial methods for hydrogen production are highly energy intensive, environmentally malign due to greenhouse gases (CO_x, NO_x and SO_x) emissions and fossil fuel dependent, thus finite. For this reason, alternative methods that are clean and renewable are being investigated. The use of biomass for sustainable hydrogen production is receiving much attention because of its renewability. Hydrogen production through thermochemical processes like biomass pyrolysis and gasification has been shown to be feasible, but has not been demonstrated at large-scale production levels (Ni *et al*., 2006).

On the other hand, biological hydrogen production processes such as photofermentation offer an opportunity to use the available renewable resources. They are mostly carried out at ambient temperatures and pressure hence less energy intensive and can utilize various renewable waste materials (Das and Veziroğlu, 2001).

2.1.3 Current Cost of Hydrogen Production

The cost of hydrogen is dependent on the cost of feed and the capital investment. Current available technology for large scale hydrogen production from fossil fuels (natural gas, oil and coal) make it cheaper compared to production from renewable sources. The production cost of large scale hydrogen production from natural gas is estimated to be 6 Euro/GJ while that of biological hydrogen production is three to four times. Technological improvements through continued research and development are expected to reduce production costs from renewable resources (Reith *et al*., 2003). The HYVOLUTION project focuses on the combination of thermophilic fermentation and photofermentation to enable the complete conversion of biomass to hydrogen. It aims to develop industrial hydrogen production at 10 Euro/GJ. This will be achieved by reducing costs in biomass treatment, optimizing efficiencies in both thermophilic and photofermentative systems, low-gas upgrading procedures and optimizing energy and heat utilization systems (Claassen and Vrije, 2006).

2.2 Biological Hydrogen Production

In nature, micro-organisms such as algae, cyano-bacteria and photosynthetic bacteria can utilize renewable resources (sunlight, water and biomass) to produce hydrogen as a by-product during growth.

Biological hydrogen production can be divided into four main categories (Das and Veziroğlu, 2001):

a. Biophotolysis of water using algae and cyanobacteria.
b. Fermentative hydrogen production from organic compounds.
c. Photodecomposition of organic compounds by photosynthetic bacteria.
d. Combined systems (Hybrid) using photosynthetic and fermentative bacteria.

2.2.1 Biophotolysis

Biophotolysis is the biological splitting of water into hydrogen and oxygen. Microalgae and cyanobacteria have been shown to be able to produce hydrogen by water photolysis using light as energy source under anaerobic condition (Gaffron and Rubin, 1992; Smith *et al*., 1992). These photosynthetic organisms have nitrogenase and hydrogenase enzymes that catalyse the water decomposition. The biophotolysis reaction is shown in Equation (2).

$$H_2O\,(l) \rightarrow \; H_2(g) + \frac{1}{2}O_2(g) \qquad (2)$$

Although biophotolysis has been shown to be a good biological system for producing hydrogen, a major obstacle is that the generated oxygen tends to inhibit the hydrogen production enzymes leading to low production rates (Benemann *et al*., 1973). Other problems include intrinsic limitations in light conversion efficiencies and problems with gas capture and separation (Hellenback *et al*., 2002; Melis, 2002).

2.2.2 Dark Fermentation

In fermentative hydrogen production, anaerobic bacteria produce hydrogen by decomposing organic substrates in the absence of light. Hydrogen, carbondioxide and lower molecular weight organic compounds such as acetate and butyrate are the main products. The lower molecular weight organic acids are a result of incomplete decomposition. Shown in Equation (3) is a fermentation reaction of glucose.

$$C_6H_{12}O_6\ (l) \rightarrow H_2(l) + CO_2(l) + \text{Organic acids} \qquad (3)$$

The amount of hydrogen production by dark fermentation depends on the chemical composition of the feed, pH value, hydraulic retention time (HRT) and the gas partial pressure. Production of alcohols (ethanol) as end products in the fermentation lowers hydrogen productivity. pH in the range of 5-6 is suggested and low hydrogen partial pressures 10–20 kPa have been shown to enable bacterial growth in dark fermentative trickle bed reactors (Fang *et al*., 2002; Groenestijn *et al*., 2002). Fermentative hydrogen production can take place between 30-80^{0}C. Studies on hydrogen production through dark fermentation using thermophilic species are becoming common because of the advantages of using high temperatures. Thermophiles are microorganisms that live at relatively high temperatures, between 45 and 80 °C. It was observed that thermophiles had higher hydrogen yields, close to the theoretical value of 4 mol H_2/mol glucose compared to their mesophilic counterparts that had 2–3 mol H_2/ mol glucose yields (Kleerebezem and Loosdrecht, 2007 ; Kengen *et al*., 2008).

2.2.3 Photofermentation

Photodecomposition of organic compounds by photosynthetic bacteria is considered the most favorable method for biological hydrogen production. The bacteria produce hydrogen by breaking down organic acids into hydrogen and carbondioxide using sunlight energy and the nitrogenase enzyme. The reaction is shown in Equation (4).

$$\text{Organic acids} + 6H_2O \rightarrow 12H_2(g) + 6CO_2(g) \quad (4)$$

The photosynthetic bacteria are reputed to have high conversion efficiencies, show versatility in using different substrates, survive in different environments (aerobic, anaerobic, with or without light and salty waters) and can trap energy at a wide range of light spectrum. Also, large database is available for genetic manipulation and enhancing hydrogen production mechanism (Fascetti and Todini, 1995, Miyake and Kawamura, 1987).

2.2.4 Integrated Systems

Dark fermentation and photofermentation hydrogen production can be combined to achieve maximum conversion of biomass and for high hydrogen yield. In dark fermentation, due to thermodynamic limitations, the end products include hydrogen, carbondioxide and lower molecular organic acids. On the other hand, nearly stoichiometric conversion of some substrates (organic acids) to hydrogen can be obtained through photofermentation (Chen *et al*., 2007). A system in which the low molecular organic acid produced in the dark fermentation is used as feed to the photofermentation process would lead to complete utilization of the initial substrate for hydrogen production.

Considerations to be made in developing the integrated system include the feed, organisms, end products, materials and methods of operation. Employment of batch tests of dark-fermentation using microflora and photofermentation with *Rhodobacter sphaeroides* SH2C led to an increase in hydrogen yield from 3.67 mol H_2/mol sucrose in dark fermentation to 6.63 mol H_2/mol sucrose using the two-step process (Tao *et al*., 2007). Results demonstrated that the combination of dark and photofermentation systems was effective and efficient in improving hydrogen yield from glucose by immobilized *Rhodopseudomonas faecalis* RLD-53 using soluble metabolites from dark fermentation with *Ethanoligenens harbinese* B49. Maximum hydrogen yield at 9 g/l glucose was increased from 1.83 mol H_2/mol glucose to 6.32 mol H_2/mol glucose (Liu *et al*., 2008). Reviews and evaluations show that

the combined system shows greater promise for industrial application (Redwood *et al*., 2008).

As stated in the introduction section, the European Union 6th framework integrated project ‘‘HYVOLUTION’’ aims to develop a two-stage bioprocess for the cost-effective production of pure hydrogen at small scale from locally obtained biomass feedstock. Intermediate products from thermophilic fermentation are to be used as feedstock in photoheterotrophic fermentation to attain an overall 75% hydrogen production efficiency.

Thermophilic fermentation that takes place at or more than 70^{0}C is preferred because of its higher hydrogen yield compared to its mesophilic counterpart. Over 3 moles of hydrogen and 2 moles of acetic acid are produced from thermophilic fermentation of glucose while 1–2 moles of hydrogen production and reduced by-products like butyrate, propionate and ethanol is obtained in mesophilic fermentations at ambient temperatures (Vrije *et al*., 2003). Also, the production of acetate, a prime substrate for photoheterotrophic bacteria makes thermophilic fermentation more feasible for integration with photofermentation process. In the project, a thermophile, *Caldicellulosiruptor saccharolyticus*, is used as the key microorganism for the dark fermentation process because of its superior characteristics for hydrogen production. It grows optimally at 70ºC, can utilise many different carbohydrates including cellulose, xylan and pectin) and it produces high levels of hydrogen (Rainey *et al*., 1994; Kadar *et al*., 2003; Kadar *et al*., 2004; van Niel *et al*., 2002). Moreover, it can simultaneously convert C_6- and C_5- sugars, the main constituents of commonly used biomass feedstock in the project; potato steam peels and sugar- beet thick juice molasses. A trickling bed and fluidized bed reactor are used in the dark fermentation process (Groenestijn *et al*., 2006; Zhang *et al*., 2006; Groenestijn *et al*., 2009).

In the photofermentation process, the PNS bacteria *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* which showed good growth and hydrogen production on acetate are used. Hydrogen production using the

effluent from the dark fermentation process is done in flat panel and tubular photobioreactors.

A schematic of the combination of thermophilic and photo fermentation concept of the HYVOLUTION integrated project is given in Figure 2.1.

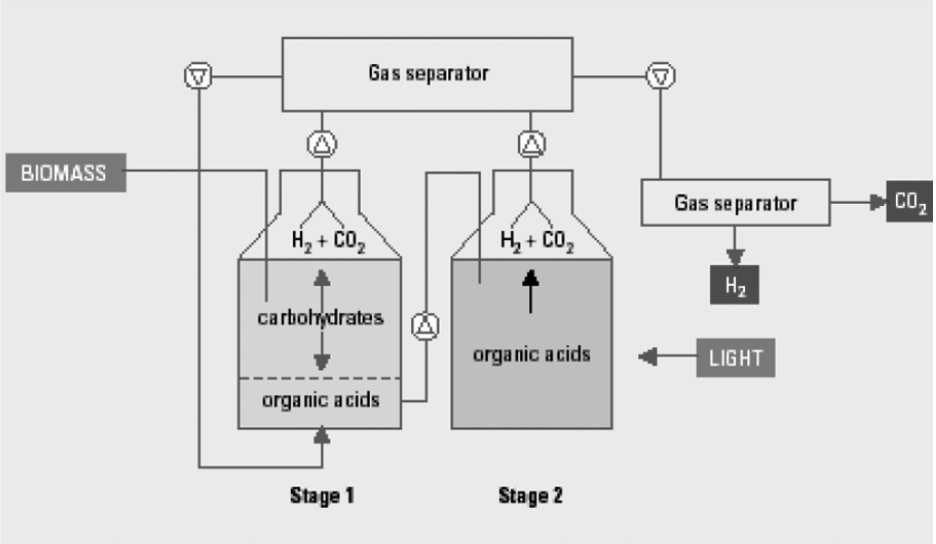


Figure 2.1 Hydrogen production from combined thermophilic and photoheterotrophic fermentation (HYVOLUTION Integrated Project concept) (Reith *et al*., 2003).

2.3 General Characteristics of *Rhodobacter capsulatus*

Rhodobacter capsulatus is a gram-negative purple non- sulphur (PNS) bacterium. It is a member of the *Rhodospirillaceae* family and belongs to the *Rhodobacter* genera. It has a rod-like shaped cell with a diameter of 0.5-1.2 μm and divides by binary fission, producing capsules and slime (Imhoff *et al*., 1995). The cells are motile by polar flagella. Shown in Table 2.1 is a classification table of the *Rhodobacter* species.

Table 2.1 Classification of *Rhodobacter capsulatus* (Imhoff *et al*., 1984).

Super Kingdom	*Prokaryota*
Kingdom	*Monera*
Sub kingdom	*Eubacteria*
Phylum	*Gracilicutes*
Class	*Photosynthetic eubacteria*
Order	*Rhodospirillates*
Family	*Rhodospirillaceae*
Genus	*Rhodobacter*
Species	*capsulatus*

Purple non sulphur (PNS) bacteria are prokaryotic and photosynthetic organisms that have a single photosystem (lack photosystem II), thus can carry out anoxygenic photosynthesis. They require vitamin for growth and are able to perform different growth modes such as, aerobic respiration, anaerobic respiration, and photosynthesis. They grow at pH 6-9 and temperature range 25^0C-35^0C (Sasikala *et al*., 1993). Also, they can live in both dark and light conditions and can tolerate oxygen at low levels (Biebl and Pfennig, 1981). The bacteria are pigmented with bacteriochlorophyll *a* and carotenoids of the sphaerodene series. *Rhodobacter capsulatus* bacteriochlorophyll *a* has characteristic absorption maxima values 376-378, 450-455, 478-480, 508-513, 590-592, 802-805 and 860-863 nm for living cells. Shown in Appendix D is its light absorbtion spectrum. The PNS bacteria exhibits a yellowish brown to greenish and deep brown colour when grown anaerobically in the presence of light but turn red in the presence of oxygen; carotenoids are converted to corresponding ketocarotenoids that cause the red colour change (Pellerin and Gest, 1983). A microscopic picture of the *Rhodobacter capsulatus* bacterium is shown in Figure 2.2.

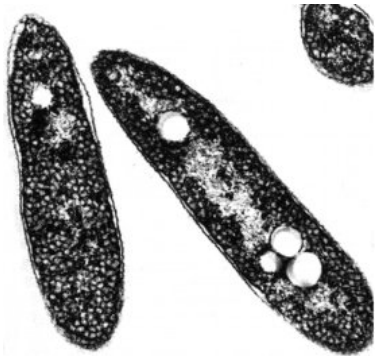


Figure 2.2 Microscopic picture of *Rhodobacter capsulatus* bacterium (Bacterial Photobiotechnology Group, Duesseldorf University).

2.4 Overview of the Hydrogen Production Metabolism

Rhodobacter capsulatus and other PNS bacteria produce molecular hydrogen under anaerobic and nitrogen-limiting conditions by using light as primary energy source and organic compounds as carbon source. The nitrogen-limiting condition (brought about by having a high C/N ratio) forces the bacteria to 'dump' the excess energy and reducing power through the production of hydrogen. The overall hydrogen production mechanism comprises several individual components: the enzyme systems (nitrogenase and hydrogenase enzymes), the TCA cycle (carbon flow) and the photosynthetic membrane apparatus (Koku *et al*., 2002). Shown in Figure 2.3 is the overall scheme for hydrogen production.

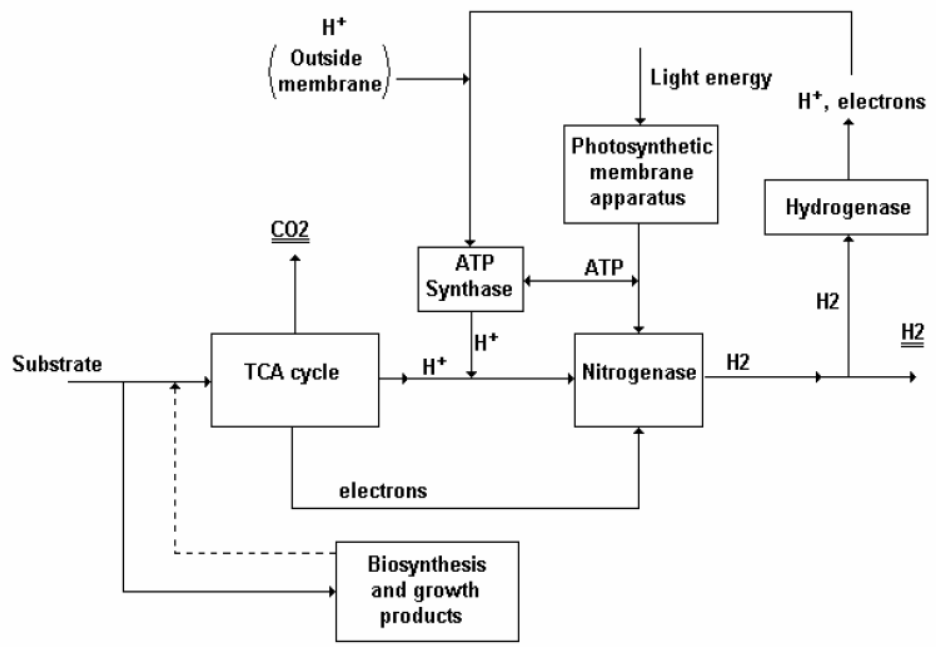


Figure 2.3 The overall scheme for hydrogen production by PNS bacteria (Koku *et al*., 2002).

2.4.1 The Hydrogen Production Mechanism

In the hydrogen production mechanism, substrates (organic acids such as acetate), are oxidized in the TCA (Tricarboxylic or citric acid cycle) to release carbondioxide, protons and electrons. A simplified diagram of the carbon metabolism is given in Figure 2.4.

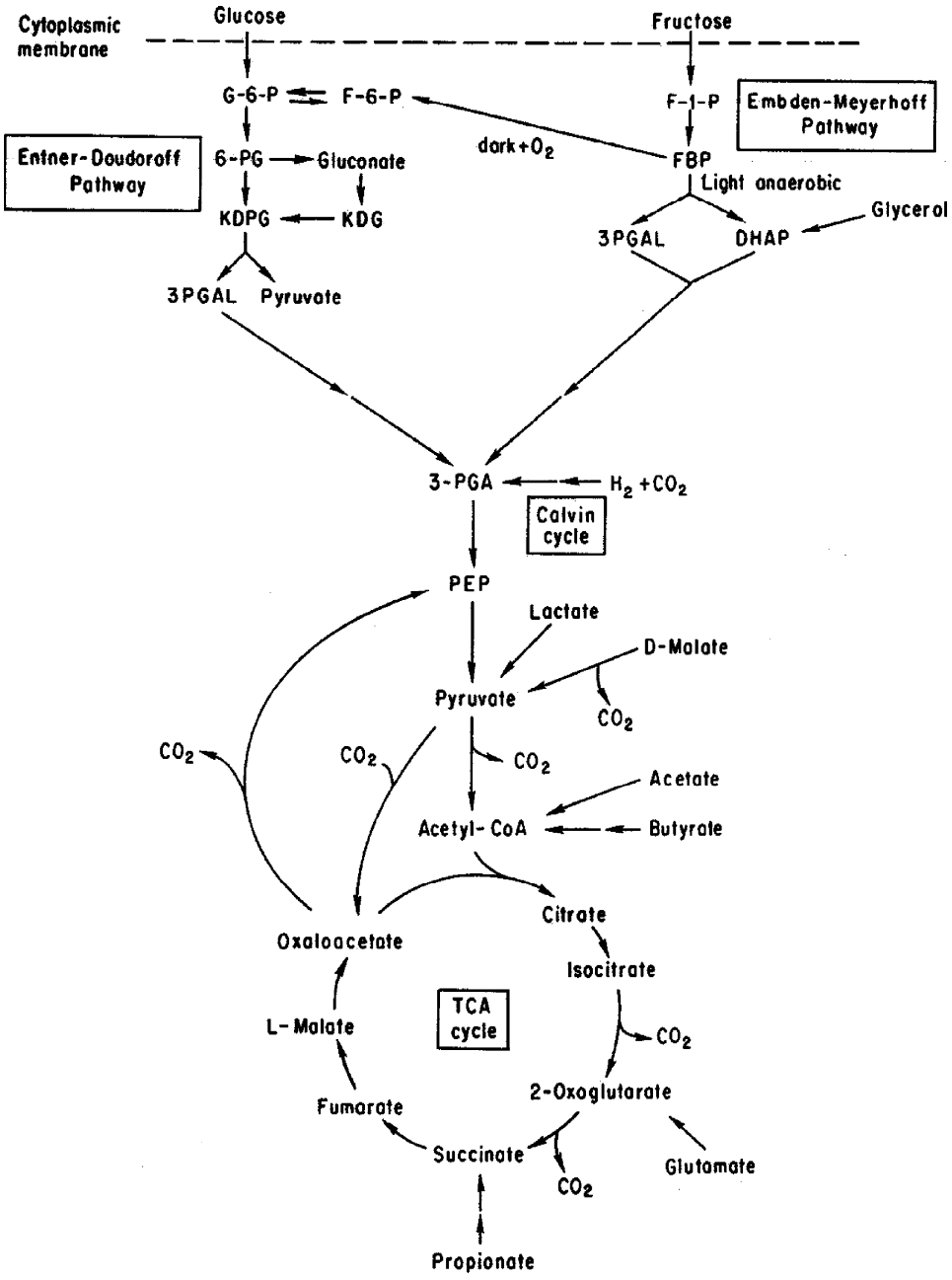


Figure 2.4 A simplifed overall scheme of the carbon metabolism in PNS bacteria (Koku *et al*., 2002).

The protons and electrons produced in the TCA cycle are directed to the nitrogenase enzyme. The photosynthetic membrane apparatus converts light energy into ATP, which is also directed into the nitrogenase enzyme. The protons to the nitrogenase enzyme are partly supplied by the action of ATP-synthase, working as a part of photosynthetic apparatus. The nitrogenase enzyme reduces the protons to molecular hydrogen (Sasikala *et al*., 1990). However, hydrogenase enzyme functions primarily in the direction of hydrogen consumption by producing ATP, protons and electrons. Therefore, the net collected hydrogen amount is the amount produced by nitrogenase minus the amount consumed by hydrogenase (Vignais *et al*., 1985).

2.4.2 Enzyme Systems

Two key enzymes, nitrogenase and hydrogenase enzymes, are involved in the hydrogen production process.

2.4.2.1 Nitrogenase

Hydrogen production is mainly attributed to the nitrogenase enzyme. Under anaerobic conditions, in the presence of molecular nitrogen, nitrogenase enzyme catalyzes nitrogen fixation by the following reaction (Miyake *et al*., 1989).

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i \quad (5)$$

In the absence of molecular nitrogen, the enzyme converts protons to hydrogen by the reaction below.

$$2H^+ + 2e^- + 4ATP \rightarrow H_2 + 4ADP + 4Pi \quad (6)$$

The nitrogenase enzyme is inhibited by oxygen and ammonium. Oxygen denatures MoFe and Fe proteins in the enzyme complex irreversibly (Hall *et al*., 1995). High ammonia concentrations repress nitrogenase expression and activity, but it recovers once the excess ammonia concentrations decrease (Gogotov, 1986; Zorin, 1986). Experiments using continuous cultures of *Rhodopseudomonas capsulatus* have shown that light

strongly stimulate nitrogenase activity (Jouanneau *et al*., 1985). Also, stable nitrogenase activity was observed with alternating ligt and dark periods (Meyer *et.al*., 1978).

2.4.2.2 Hydrogenase

Two types of hydrogenase enzymes are involved in the hydrogen metabolism. The first type, designated as ‘‘reversible’’ hydrogenase is presumed to catalyze hydrogen evolution or uptake depending on the redox status of the culture. ‘‘Uptake’’ hydrogenase is the second type of hydrogenase enzyme. It is membrane bound and primarily catalyzes hydrogen consumption (Hall *et al*., 1995).

The hydrogenase enzyme catalyzes the reversible breakdown of molecular hydrogen according to the reaction shown in Equation (7).

$$H_2 \leftrightarrow 2H^+ + 2e^- \quad (7)$$

Studies using *R. capsulatus* have shown that the hydrogenase enzyme is able to produce and consume hydrogen. However, since hydrogen production is mainly attributed to the nitrogenase enzyme, its hydrogen-producing activity is neglible. Hydrogenase activity was observed to be less than 10% of the hydrogen consuming activity (Gogotov, 1986; Zorin, 1986).

Repression of the hydrogenase enzyme through genetic modifications like chromosomal inactivation of the uptake hydrogenase enzyme can increase hydrogen production. Two mutants of *R. capsulatus* B10, lacking the uptake hydrogenase enzyme (Hup^-) were grown in a medium containing lactate and glutamate. They produced 10-20% more hydrogen at higher rate than their wildtype counterpart Zorin (1996). 68% hydrogen yield was obtained from 60 mM malate feed using *R. capsulatus* ST410 (a Hup^- mutant) compared to 25% hydrogen yield in the wildtype *R. capsulatus* B100 (Ooshima *et.al*., 1998). Öztürk *et al*. (2006) developed a mutant strain (Y03) of *R. capsulatus* MT1131 by genetically deleting the uptake hydrogenase through interposon mutagenesis. They observed that when grown in a medium containing 15 mM

malate and 2 mM glutamate, the mutant strain had a higher hydrogen productivity and substrate conversion efficiency compared to the wild type strain. The mutant strain produced a total of 1.33 ml of hydrogen per ml culture with a maximum production rate of 0.019 ml H_2/ml culture.h and 67% substrate conversion efficiency while the wild type strain produced a total of 0.94 ml of hydrogen per ml culture with a maximum production rate of 0.014 ml H_2/ml culture.h and 47% substrate conversion efficiency.

2.4.3 By-products

Production of hydrogen using photosynthetic bacteria (PNS) is accompanied by the formation of by-products. These by-products increase the added value of the overall process. Biomass is one of the by-products. Cells from photosynthetic are rich in high quality protein, biological-factors and vitamins (of the group B) (Koyabashi and Kurata, 1978). Proteins of *R. capsulata* are shown to be rich in essential sulphur and amino acids with high lysine content (5.4%), thus can be used as a complementary diet of animals because cereals are poor in lysine content Vrati (1984).

Poly-3-hydroxybutyrate (PHB) is another important by-product of the hydrogen production process. It is the simplest poly-3-hydroxyalkanoate and a biodegradable thermoplastic that is synthesized by bacteria during unfavourable growth conditions; under stress conditions during stationary phase of growth. It is used as a storage material by the bacteria and its accumulation depend on the carbon substrate, nitrogen availability and the pH of the medium. Under ammonium and nitrogen poor conditions, using acetate as carbon source, high levels of PHB were produced by *R. sphaeroides* (Krahn *et al*., 1996). The thermoplastic properties of PHB and its biodegradability make it a suitable substitute for petrochemical plastics. Also, it can be industrially used in the construction of biodegradable carriers for long-term dosages, either in the agriculture for herbicides and insecticides, or in the medical field for drugs and also for surgical sutures (Khatipov *et al*., 1998; Yiğit *et al*., 2000; Suludere, 2001).

Another significant by-product is the carotenoid pigments. They are essential in photosynthesis as they transfer nearly half of the absorbed light energy to bacteriochlorophyll. They also prevent the photoinhibitory effects of the sun on bacteria and enable photosynthesis to occur under oxygenic atmosphere. They are a class of hydrocarbons (carotene) and their oxygenated derivatives (xanthophylls). Carotenoids have been used commercially during cancer chemoprevention, as a food colorant, natural antioxidant and provitamin A source.

2.5 Acetate as a Substrate

PNS bacteria can utilize different low molecular weight organic acids such as for hydrogen production under anaerobic conditions. As discussed in the hydrogen production metabolism (Section 2.4.1), organic acids such as malate, lactate, butyrate and acetate act as carbon sources (electron donors). Shown below are equations relating to conversion of some organic acids to hydrogen and carbondioxide.

$$\text{Lactate: } C_3H_63O_3 + 3H_2O \rightarrow 6H_2 + 3CO_2 \quad (8)$$

$$\text{Malate: } C_4H_6O_5 + 3H_2O \rightarrow 6H_2 + 4CO_2 \quad (9)$$

$$\text{Butyrate: } C_4H_88O_2 + 6H_2O \rightarrow 10H_2 + 4CO_2 \quad (10)$$

$$\text{Acetate: } C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2 \quad (11)$$

A substrate conversion efficiency of 20-100% from organic acids to hydrogen was shown among purple non-sulfur bacteria (Segers *et al*., 1983; Stevens *et al*., 1986). Nitrogen sources like glutamic acid contain carbon and hydrogen that can be utilized by the photosynthetic bacteria. This would lead to substrate conversion efficiencies of over 100%. Sasikala *et al*. (1990), observed over 100% substrate conversion efficiency using *R. sphaeroides* and malate as feed. They attributed this to the existence of endogenous substrates which acted as electron donors for hydrogen production. Endogenoous substrates are known to act as electron donors for hydrogen formation (Ormerod *et al*., 1961; Hillmer and Gest, 1977). In this study, calculations on the substrate conversion

efficiencies were made with regards to acetate and glutamate. Glutamate contains five carbon atoms per nitrogen atom and so is a significant additional carbon source for the bacteria at high concentrations.

The use of expensive synthetic culture media used in the laboratory experiments is not practical and cheap organic substrates must be used. These are mostly residual wastes from the food industry or waste water with high levels of organic compounds (Rocha *et al*., 2001). Several studies have been done on hydrogen production using PNS bacteria from wastes; ranging from starch, sewage sludge, whey, waste water of milk, vegetable juice and olive mill wastes (Sasikala *et al*., 1991; Türkaslan *et al*., 1998; Zhu *et al*., 1999; Yetiş *et al*., 2000; Eroğlu, 2006). However, major components of waste waters are carbohydrates and proteins but photosynthetic bacteria can more effectively use low molecular organic compounds as carbon sources. The combination of dark and photo fermentative hydrogen production is a proposed solution (Mao *et al*., 1986; Sasaki *et al*., 1998). Low organic substrates produced in the dark fermentation are utilised in the photo fermentation.

Malate and lactate have been reported as the most favorable substrates giving the highest hydrogen production (Hillmer and Gest *et al*., 1977, Kim *et al*., 1980; Miyake *et al*., 1984, Sasikala *et al*., 1997; Eroğlu *et al*., 1999). However, little is known about the conversion of acetic and butyric acids to hydrogen by PNS bacteria (Segers *et al*., 1983; Sasaki *et al*., 1998). Enzymes for the glyoxylate cycle were found to be present when *R. capsulatus* was grown in acetate containin medium (Fuller *et al*., 1961, Wilson *et al*., 1988).

Acetate is a prime substrate for photoheterotrophic bacteria because of several reasons. Firstly, energy from light enables photoheterotrophic bacteria to overcome the thermodynamic barrier in the conversion of acetic acid to hydrogen (Akkerman *et al*., 2002). Acetate is a central intermediate in the overall carbon cycle and it enters the central metabolism at the level of acetyl-coenzyme A. Acetyl-CoA, a thioester between Coenzyme A (an acyl group carrier) and acetic acid, is a very important molecule in the TCA cycle as its main task is conveying the carbon atoms within the acetyl group to the citric

acid cycle to be oxidized for energy production. Also, acetate and butyrate are the dominant products of the dark fermenataion process (Segers *et al*., 1981). Therefore, the use of acetate enables a possibility of integrating dark fermentation with photofermentation to achieve high hydrogen production. Moreover, acetate is cheaper and available compared to other substrates like malate.

There are several studies done on hydrogen production from acetate by photosynthetic bacteria. Kuriuki *et al*. (1998) investigated hydrogen production from batch cultures of *Rhodobacter sphaeroides* RV with acetate, propionate, lactate, butyrate and valerate. Despite being completely utilised, no hydrogen production was observed. This indicated that it was converted to other component such as PHB.

Rocha *et al*. (2001) obtained maximum hydrogen production rate at 25 ml H_2/l.h on acetate using *Rhodopseudomonas sphaeroides*. A decrease in acetate concentration from 22 to 11 mM resulted in a decrease in the hydrogen evolved from 214 to 27 ml H_2 per vessel.

Shi and Yu (2006) studied hydrogen production by *Rhodopseudomonas capsulata* using acetate, propionate and butyrate, and a mixture of the three organic acids (1.9 g/l acetate, 0.4 g/l propionate and 0.8 g/l butyrate. Highest hydrogen yield of 0.65 and maximum hydrogen production rate of 17.7 ml/l.h was obtained using acetate feed. An increase in acetate and propionate concentration led to an increase in hydrogen production. Hydrogen yield of 0.34 and maximum production rate of 14.5 ml/l.h was obtained in the combined organic acid mixture. The consumption order acetate>propionate>butyrate was obtained for the combined organic acid mixture.

Asada *et al*. (2008) investigated hydrogen productivity from acetate by agar-immobilized photosynthetic bacteria under illuminated conditions. Among the five tested strains, *Rhodobacter sphaeroides* RV gave highest yield hydrogen yield of 2.81 mol H_2 per mol of acetate using 21mM acetate. They

observed that lower hydrogen yield of 2.65 mM mol H_2 per mol of acetate using higher (42 mM) acetate concentration.

Özgür *et al*. (2009) tested *Rhodobacter capsulatus* on 10-50 mM initial acetate concentrations and 2 mM glutamate in 50 ml bottles set at 30^0C and 200W/m^2 illumination. The highest hydrogen production of 0.7 mmol/l_c.h was obtained with 40 mM initial acetate concentration.

2.6 Photobioreactors

Photobioreactors (PBR) are enclosed transparent reactors in which light can pass and are used to grow microorganisms or carry out photobiological reactions (Tredici, 2004). They can be classified as panel or tubular with regards to geometry and batch or continuous with respect to the mode of operation. They can be horizontal, vertical, inclined, manifolded or spiral and can be operated with or without mixing.

2.6.1 Types of Photobioreactors

Different types of photobioreactors have been designed and used for hydrogen production using photosynthetic bacteria. Hoekama *et al*. (2002) developed a lab scale flat-panel photobioreactor for anaerobic cultivation of PNS bacteria *Rhodopseudomonas sphaeroides* HCC 2037 and the production of hydrogen from succinate and acetate. Tredici *et al*. (1997) cultivated *Arthrospira platensis* (cyanobacterium) in outdoor conditons in a 32 l near horizontal tubular reactor. Eroğlu *et al*. (1999) investigated hydrogen production using malate and glutamate as carbon and nitrogen sources in a 400 ml batch water jacketed glass column photobioreactor with *Rhodobacter sphaeroides* O.U.001. Tsygankov *et al*. (1993) fabricated photosynthetic bacteria immobilized on porous glass for hydrogen production. Modigell and Holle (1998) investigated hydrogen production in a hollow channel plate PBR made of acrylic glass.

Phototrophic microorganisms are highly diverse in their morphology, nutritional and light requirements and resistances to stresses. For this reason,

PBRs cannot be designed to fit all their conditions (Tredici *et al*., 2004). However, the development of a PBR, which ensures a simple design, low material and production costs and high utilization of sunlight within the optimum wavelength range, is targeted for sustainable hydrogen production. The modes of operation, size, geometry and the position (orientation) of the photobioreactor are the most important design parameters (Wakayama *et al*., 1998; Tredici *et al*., 1999). Photobioreactors with increased surface-to-volume (S/V) ratio built by applying flat-plate or tubular types can utilize irradiated light energy efficiently (Nakada *et al*., 1995; Ogbanna *et al*., 1995).

Flat panel photobioreactors facilitate the measurement of irradiance at the culture surface hence are commonly used in the laboratory (Tredici, 2004). They consist of a rectangular transparent box with a depth of 1-5 cm. The height and width can be varied and so far panels with height and width less than 1 m have been studied (Akkerman *et al*., 2002). An advantage of the flat panel PBRs is that they can be tilted (oriented) at optimal angles for maximum exposure to direct light (Tredici and Zitelli, 1997; Richmond *et al*., 1999). Also, they can be arranged in a stack close to each other therefore providing a large illuminated area in a small ground area. The packed arrangement allows a five-fold dilution of solar radiance at the surface, an effect called lamination, which greatly enhances the efficiency of conversion of solar radiance into biomass (Carlozzi, 2000; Richmond and Zhang, 2001). Temperature distribution experiments demonstrated that a 25 cm gap between the flat panel PBRs was optimal for hydrogen production. With the 25 cm spacing, the maximum temperature of the culture remained close to the physiological limit (32^0C) at the occurrence of the highest day time temperature (Gebicki *et al*., 2007). Moreover, the flat panel PBRs are flexible and easy to scale-up.

A disadvantage of flat panel PBRs is the lack of mixing. Mixing ensures uniform distribution of cells and feed medium, thus enabling efficient growth and utilization of substrates leading to high productivity. Zabut *et al*. (2006) observed that stirring increased the total gas produced and enhanced hydrogen production rate in 400 ml water-jacketed-glass column

photobioreactor. However, mixing can be achieved in these systems using gas introduced via perforated tube at the bottom and bubbled into the reactor (Akerman *et al*., 2003).

Arai *et al*. (1998) investigated hydrogen production by *Rhodobacter sphaeroides* RV using lactate and propionate as carbon sources. Hydrogen production rate of 12.5 ml H_2/l_c.h was obtained in a 4.4 l photobioreactor made of acrylic resin.

Nakada *et al*. (1998) examined hydrogen evolution at various light penetration depths in flat panel PBR using *R. sphaeroides.* The photobioreactor comprised four compartments (each 5mm apart) of bacterial suspension. They found that 69% of the incident light energy was absorbed in the first compartment (0-5mm) and 21% was absorbed in the second (5–10 mm).

Zhang *et al*. (1999) evaluated the ability of a photobioreactor to fix CO_2 using the thermophilic cyanobacterium, *Synechocystis aquatilis* SI-2. They also investigated the effects of orientation of the vertical flat panel PBRs made of transparent acrylic plastic and observed that an east-west facing orientation had 1.4 fold higher productivity than a north-south orientation. This was because more solar energy was received in the former orientation.

Ogbonna *et al*. (1999) installed a device for collecting solar light and distributing it inside a stirred bioreactor through optical fibers. A light tracking sensor that rotated the lenses with the position of the sun was set up. The system was integrated with the electric gridline and used to cultivate *C. sorokiniana* IAM C-212. The stirred tank agitation speed and cultivation temperature were 120 rpm and 36°C respectively. An average linear growth rate of 0.11 g/l.day during cloudy days and at night was obatined. This value was still lower than the growth rate obtained on sunny days (0.3 g/l.day), but it provided a solution to the problems of night biomass loss and low productivity on cloudy days.

Uyar *et al*. (2007) studied the effect of light intensity and light wavelength on the growth and hydrogen production of *R. Sphaeroides* O.U. 001 in gas-tight glass photobioreactors with defined medium. It was

found that the rate of hydrogen production increased with increasing light intensity and reached saturation at around 270W/m^2. Also, the lack of infrared light (750–950 nm wavelength) decreased photoproduction of hydrogen by 39%.

Eroğlu *et al*. (2008) investigated the performance of an 8 l flat plate solar bioreactor operating in outdoor conditions. They used different organic acids (malate, lactate and acetate) and olive mill waste as carbon sources for *R. sphaeroides* O.U.001. Highest hydrogen production (0.01 l/l/h) was obtained with malate and formate formation was observed as the fermentation end product. Acetate resulted in low hydrogen gas production and high PHB accumulation.

2.6.2 Materials of Construction

Materials of construction of photobioreactors should be transparent to allow light to pass and be utilized by the microorganism for growth and hydrogen production. The materials should also lack toxicity. Chemically stable (unreactive) and metal free materials are recommended because some metals like nickel and iron are known to activate hydrogenase enzyme and inhibit hydrogen production. In addition, the materials should be durable, have high mechanical strength, be easy to clean and available at low cost. Several materials for constructing PBRs are discussed.

Glass is considered a very good material for the construction of panel PBRs because of its transparency, low hydrogen permeability and long lifespan (10 years). However, it is brittle, rigid, heavy and not workable, thus not suitable for large scale systems (Burgess *et al.*, 2004).

Polymethyl methylacrylate (PMMA or Pleksiglas) is another suitable material for panel PBR construction. It is highly transparent thermoplastic with a density of 1,150–1,190 kg/m^3; less than half the density of glass. It is soft and easy to work with. It transmits 92% of visible light (3 mm thickness) and filters ultraviolet (UV) light wavelengths below 300 nm while allowing infrared light of up to 2800 nm wavelength to pass. Also, it is weather resistant and can

withstand outdoor conditions better compared to other plastics such as polycarbonate. However, it is brittle, inflexible and has higher hydrogen permeability compared to glass. A wall thickness of 4 mm minimum is needed to avoid leakage and cracking due to mechanical stress. It has a shorter lifespan (1-2 years) compared to glass (Burgess *et al*., 2004).

Low density polyethylene (LDPE) is a flexible thin material. It is widely used in greenhouse covering, which has similar requirements as the PBR; high visible light and near infrared transmission, low UV transmission and low cost (Hussain *et al*., 2004). A major disadvantage of the LDPE is that with the reduced wall thickness, hydrogen permeability through the walls gains influence on the gas collected. Also, it has a short lifespan (1 year) (Burgess *et al*., 2004).

2.7 Ammonium Ion Removal Using Clinoptilolite Zeolite

Ammonium ion is an inhibitor to the nitrogenase enzyme of PNS bacteria such as *Rhodobacter capsulatus* as described in Section 2.4.2. High ammonia concentrations repress nitrogenase expression and activity, but it recovers once the excess ammonia concentrations decrease (Gogotov, 1986 and Zorin, 1986). Hydrogen production by *R. capsulatus* grown on acetate and NH_4Cl as carbon and nitrogen sources was tested at different ammonium chloride concentrations; feed medium containing 30 mM acetate and 1 mM, 2 mM, 4 mM and 8 mM NH_4Cl was used. Biomass increase with increasing NH_4Cl concentration was observed. pH also increased with increasing biomass and hydrogen production decreased. Significant hydrogen production was observed at 1 mM NH_4Cl (Uyar, 2008).

The DFE effluent from the dark fermentation process contains high amounts of ammonium (9-12 mM) that is not suitable photo fermentation process. To be able to utilize the DFE, it was proposed to reduce the ammonium concentration in the DFE using clinoptilolite zeolite.

Clinoptilolite Zeolite

Clinoptilolite is a natural zeolite with microporous arrangement of silica and alumina tetrahedral channels contains exchangeable cations such as K^+, Na^+, Mg^{2+}, Ca^{2+}.[1] It is a silica rich member of heulandite family of zeolites and has Si/Al ratio of greater than 4. It has the structure $(Na, K, Mg)_6(Al_6Si_{30}O_7)\cdot 24H_2O$ and is mostly enriched with potassium and sodium ions (Breck, 1971; Szostak, 1984). It is the most abundant natural zeolite and is found with high purity in large mineable quantities, in different parts of the world (Mumpton, 1978; Ouki *et al*., 1994; Sarıoğlu, 2005). In Turkey, there are large reserves of clinoptilolite and other zeolites. Clinoptilolites deposits can be found in Bigadiç, Balıkesir and Gördes, Manisa. Surveys have shown that about 20 million tons of Gördes clinoptilolite are available (Moralı, 2006).

Clinoptilolite is known to have high selectivity for certain cations; Pb^{+2}, NH_4^{+1}, Cd^{+2} , Co^{+2} Cr^{+2}, Fe^{+2}, Cs^{+1} and Na^{+1}. Due to its affinity for heavy metals, it has been investigated by many researchers for removal of various heavy metal ions and ions in waste water (Semmens and Seyfarth, 1978; Jama and Yücel, 1989, Zamzow *et al*., 1990; Ouki and Kavannagh, 1999, Abusafa *et al*., 2002; Çulfaz and Yağız, 2004, Inglezakis and Grigoropoulou, 2004). Gördes clinoptilolite was shown to have $Pb^{+2} > NH_4^{+1} > Na^{+1}$ (Aşiroğlu, 2006). Applications of clinoptilolite have been mainly in the removal of heavy metals like Pb^{2+} and Cd^{2+} of ions, radioactive $^{137}Cs^+$ and $^{90}Sr^{2+}$ and ions (NH_4^+) from waste water streams.

Clinoptiloite exists in two forms; natural and synthetic. The natural form is highly selective but has a low ion exchange capacity while the synthetic one has a high ion exchange capacity but a low selectivity. In this study, natural Gördes clinoptilolite is used. Before usage, pretreatment of the zeolite was mandatory. Pretreatment improves the zeolite's ion exchange ability by replacing the exchangeable cations in the zeolite with more easily removable ones (Inglezakis *et al*., 2001). The Gördes clinoptilolite was batch

pretreated. Shown in Appendix J are the chemical composition and a scanning electron micrograph of the original Gördes Clinoptilolite.

2.8 Objectives of This Study

Studies done by the METU Biohydrogen Group showed that *Rhodobacter capsulatus* (DSM 1710) had a better stability and performance in acetate containing media, compared to the other PNS bacteria the group worked on; *Rhodobacter sphaeroides* and *Rhodospirillum rubrum*. Good growth and high hydrogen production was obtained in batch experiments with feed medium containing 40 mM acetate and 7.5 mM lactate as carbon sources and 2 mM glutamate as nitrogen source (Uyar, 2008). Further indoor experiments using *Rhodobacter capsulatus* (DSM 1710) strain showed that the removal of lactate from the feed did not influence hydrogen productivity significantly in indoor continuous photobioreactors (Avcıoğlu *et al*., 2009). Also, *Rhodobacter capsulatus* YO3 (Hup^-), a mutant strain lacking the uptake hydrogenase enzyme developed by the group (Öztürk, 2005), portrayed better growth and gave higher hydrogen productivities in indoor and outdoor conditions compared to the *Rhodobacter capsulatus* (DSM 1710) strain (Özgür *et al*., 2009). Moreover, during the summer of 2008, a heat adapted strain of the *R. capsulatus* (DSM 1710) was achieved after 6 generations under 40-45^{0}C daytime temperatures. The experiments on the *Rhodobacter capsulatus* strains were carried out in batch photobioreactors, so their performance in continuously operated systems remained to be assessed.

In this study, three strains of *Rhodobacter capsulatus*; *Rhodobacter capsulatus* (DSM 1710), heat adapted *Rhodobacter capsulatus* (DSM 1710) and *Rhodobacter capsulatus* YO3 (Hup^-), were tested for stable and high hydrogen production using 8 l continuous panel photo bioreactors operated in outdoor conditions. Defined hydrogen production medium containing acetate (40 mM) and glutamate (2 mM) was used as feed. The experiments were carried out between September-December 2008. Results were assessed with regards to hydrogen productivity, substrate conversion efficiency and acetate

conversion. The findings from the outdoor experiments led to further research on optimization of the feed composition for the *Rhodobacter capsulatus* YO3 (Hup^-) strain. Different feed compositions containing acetate (40-80 mM) and glutamate (2-10 mM) were examined to obtain a stable biomass and hydrogen production. The optimization of the feed in the continuous systems can help understand the relationship between cell growth, hydrogen production rate and substrate concentration which will be beneficial in developing kinetic models that can be applied in large scale hydrogen production.

Another part of this study was the reduction of ammonium ion content in the dark fermenter effluents (DFEs) using clinoptilolite zeolite. The dark fermentation process effluents contain high amounts of ammonium (9-12 mM) that is not suitable for photofermentation process. High ammonia concentrations repress nitrogenase expression and activity of the photosynthetic bacteria (Gogotov, 1986 and Zorin, 1986). No hydrogen production was observed in the DFE of sugar-beet thick juice molasses containing over 1 mM ammonium ion concentration. Therefore, it was aimed to reduce the ammonium concentration in the DFE using clinoptilolite zeolite.

Clinoptilolite is a natural zeolite with microporous arrangement of silica and alumina tetrahedral channels. It contains exchangeable cations such as K^+, Na^+, Mg^{2+}, Ca^{2+}.[1] It has a high selectivity for ammonium ions and is mostly used in the removal of heavy metals like Pb^{2+} and Cd^{2+} ions from waste water streams. Gördes clinoptilolite type zeolite was used in this study. It was pretreated using NaCl solution to enhance its ions exchange capacity. The zeolite and DFE of sugar-beet thick juice molasses were treated using batch procedure.

CHAPTER 3

MATERIALS AND METHODS

3.1 The Microorganisms

Rhodobacter capsulatus wildtype (DSM 1710), heat adapted *Rhodobacter capsulatus* (DSM 1710) and *Rhodobacter capsulatus* YO3 (Hup^-) photosynthetic bacterial strains were used in this study. *Rhodobacter capsulatus* (DSM 1710) was obtained from Deutsche Sammlung von Mikroorganismen (DSM, Braunsweig Germany). The heat adapted *Rhodobacter capsulatus* (DSM 1710) strain was obtained through six passages done on natural adaptation of the bacteria grown in outdoor conditions, under natural sunlight. The natural adaptation procedure is described in Appendix M. *Rhodobacter capsulatus* YO3 (Hup^-), a mutant strain lacking the uptake hydrogenase enzyme (Hup^-), was genetically modified by Dr. Yavuz Öztürk (GMBE, TÜBİTAK-MAM, Gebze) from *Rhodobacter capsulatus* MT1131 (Öztürk *et al*., 2006).

3.2 Culture Media

3.2.1 Growth Media

Acetate (20 mM) and glutamate (10 mM) were added to the standard basal medium (Biebl and Pfennig, 1981) as carbon and nitrogen sources respectively. The component amounts in the growth media are shown in Appendix A. The C/N ratio of the growth medium has been approved using elemental balances as shown in Appendix B. The formula for *R. capsulatus* is taken as $CH_{1.76}O_{0.38}N_{0.14}$ as suggested by Hoekama *et al*. (2002). In preparing the growth medium, the components were dissolved in distilled water and the

pH set to 6.3-6.4 by adding 5 M NaOH. The solution was then sterilized by autoclaving. Vitamin, trace elements and iron-citrate were added to the solution in a sterile cabin after autoclaving.

3.2.2 Hydrogen Production Media

Similar to the growth media, the hydrogen production media comprised the standard basal medium and acetate and glutamate as carbon and nitrogen sources respectively. Different acetate (40-80 mM) and glutamate (2-4 mM) concentrations were used in the experiments. By changing their amount, the C/N ratio is changed as shown in Table 3.1.

Table 3.1 C/N ratio constants for different acetate and glutamate concentrations.

Acetate (mM)	Glutamate (mM)	C/N Ratio
20	10	9 [a]
40	2	45 [b]
40	3	32 [b]
40	4	25 [b]
60	3	45 [b]
60	4	35 [b]
80	4	45 [b]

a) Ratio in the growth medium.

b) Ratio in the hydrogen production medium.

The hydrogen production media was prepared in the same way as the growth media, the main difference being in the amount of acetate and glutamate concentrations used.

3.3 Experimental Setup for Hydrogen Production

The hydrogen production experiments were carried out in panel photobioreactors. The 8 l photobioreactors with 0.5 m x 0.4 m x 0.04 m dimensions were made of 6 mm transparent acrylic sheet (Plexiglas). As shown in Figure 3.1, each reactor had three ports at the top and two at the side wall. The outlets at the top of the photobioreactor are for gas collection, temperature probe and feeding purposes. The side wall ports are used for sampling.

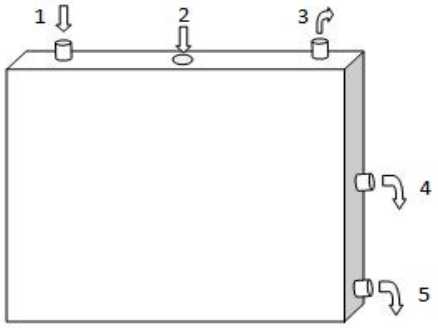


Figure 3.1 The panel photobioreactor, Port (1) medium feeding, Port (2) temperature probe, Port (3) gas collection and Port (4, 5) sampling.

3.3.1 Indoor Experiments

The indoor hydrogen production experiments were done under continuous illumination and controlled temperature. Four 60 W tungsten lamps, two placed on each side of the vertical photobioreactor, were used to provide 1500-2500 lux (86-143 W/m^2) light intensity. The room temperature was controlled using an air conditioner (Vestel SX-12 Wall split model) and the photobioreactor temperature was kept constant between 30-32 ^0C. The gas evolved was collected in a water filled graduated glass column connected to the top of the photobioreactor using polyurethane tubing. Shown in Figure 3.2 is the indoor experimental setup.

Figure 3.2 The indoor hydrogen production experimental setup using *R. capsulatus* YO3 (Hup^-).

3.3.2 Outdoor Experiments

In the outdoor experiments, the photobioreactors were operated under natural sunlight conditions. They were placed 20cm apart in an east-west facing position on a metallic construction. The east-west position was to enable optimum heat input and light intensity utilization. An online temperature probe (Elimko) connected to a computer was used to continuously measure the photobioreactor and air temperature. Gas collection was done in a water filled graduated glass column. The outdoor experiments were carried out between September-December, 2008. On the onset of winter, the photobioreactors were moved to a glasshouse. Shown in Figure 3.3 is an outdoor experimental setup.

Figure 3.3 The outdoor hydrogen production experimental setup. (1, 2) *Rhodobacter capsulatus* YO3 (Hup^-), (3) Heat adapted *Rhodobacter capsulatus* (DSM 1710) and (4) *Rhodobacter capsulatus* (DSM 1710).

3.3.3 The Glass house

The glasshouse is a green house without climatization. Its dimensions are 5 m x 4 m x 3 m and is made of transparent glass. It is connected to a container on one side in which a computer used for continuous temperature measurement is kept. During summer, the glasshouse is covered with a greenhouse material to provide shading. The shading material has been shown to pass light at wavelength between 400-900 nm and decrease light intensity by approximately 60% (Uyar, 2008). In winter, during the day, no heating or cooling is applied, but at night, when outside temperatures drop below 0^0C, the glass house is heated using a heater (Alaadin LX 2886, 2kW heater). This prevents the photobiorecators from freezing and rupturing. Shown in Figure 3.4 are the outdoor experiments in the glasshouse in winter, 2008.

Figure 3.4 Outdoor experiments in winter, 2008.

3.4 Experimental Procedure

3.4.1 Preparation of the Bacterial Inoculum

The bacterial inoculum for the hydrogen production experiments was prepared in two steps. The first step was the activation of the bacteria. The original bacteria that were glycerol stocked and stored in vials at -80^{0}C were streak plated on sterile 20 ml plates containing agar solution and 20 mM/10 mM Ac/Glu growth medium. This was done in a sterile cabin to prevent contamination. The agar plates were then placed in an incubator set at 30^{0}C and illuminated with 60W tungsten lamps. The second step was the multiplication of the inoculum. After growing for about 5 days, visible single bacterial colonies on the agar plate were transferred into 10 ml vials containing growth medium. Selection of the single colonies ensured formation of pure cultures and minimizes the risk of contamination. It took 3-4 days for the bacteria to grow to the desired mid-logarithmic phase (at around OD 1.0). Then the active bacterial culture in the 10 ml vials was injected into fresh 100 ml growth medium using sterile syringes; 10% inoculation of the active bacterial culture. After 2-3 days, when the bacteria culture was in the mid-logarithmic phase, it was inoculated to 1000 ml growth medium where it grew for 2-3 days befor inoculation to the photobioreactors. The passages of the bacteria from the 10 ml to 1000 ml were done with 10% inoculation and the bottles were flushed with argon to provide anaerobic conditions. All the inoculations were carried out under sterile conditions in a sterile cabin and using sterile syringes. The bacterial culture was grown in the incubator set at 30^{0}C and illuminated with 60W tungsten lamps.

3.4.2 Leakage Test, Cleaning and Sterilization of the Photobioreactors

The photobioreactors were tested for leakage by fully filling them with water and providing excess liquid pressure using a peristaltic pump. Leakages at the ports and sides were then checked for. After the leakage test, the photobioreactors were chemically sterilized using hydrogen peroxide solution. They were filled with 3% (w/v) H_2O_2 solution and after waiting for 24 hours, emptied and thoroughly rinsed off with distilled water.

3.4.3 The Startup Procedure

At the startup of the experiment, 25% bacterial inoculation was introduced into the photobioreactor. Leaving about 5% headspace, the 8 l photobioreactor was filled with 5.6 l of 40 mM/2 mM Ac/Glu hydrogen production medium and inoculated with 1.9 l of active bacterial culture. Then argon gas was flushed into the system to remove air and make it anaerobic. Flushing of argon also provides uniform mixing in the photobioreactors. After flushing, the photobioreactor was connected to the gas collection column and the bacterial culture was illuminated using tungsten lamps in the indoor experiments or sunlight in the outdoor experiments. The culture was left to grow and reach stationary phase (O.D≥1.5) before continuous feeding was commenced. The startup period covered the period from the photobioreactor setup and bacterial inoculation to the start of continuous feeding.

3.4.4 Operation

The photobioreactor was operated in fed-batch mode. After startup, 10% v/v daily feeding of hydrogen production medium was started when the bacterial culture reached the stationary phase (OD≥ 1.5). Liquid samples for analyses (5 ml) were taken from the side ports on the photobioreactors. During the startup, samples were taken at fixed regular intervals of 6 hours and after the start of feeding (continuous operation), sampling and feeding was done at 24 hours interval. Sampling was done before feeding the hydrogen production

medium. 800 ml bacterial effluent was removed and 800 ml hydrogen production medium was fed into the system using sterile 60 ml syringes. The bacterial effluent was chemically sterilized and discarded.

The daily analyses included cell concentration, pH, organic acid concentration, bacteriochlorophyll *a* and temperature readings. Out of the 5 ml samples taken for analysis, 1.5 ml sample was placed in eppendorfs and centrifuged at 13400 rpm for 10 minutes. The supernant was transferred to clean eppendorfs and stored at -20^0C for organic acid analysis using HPLC. The residue was discarded. Also recorded daily was the total gas produced. The gas collection columns were refilled everyday after each reading. Gas samples were analyzed using gas chromatography.

3.4.5 Shutdown

At the end of the experiments, the photobioreactor was emptied and the effluent chemically sterilized and discarded. The photobioreactor was washed with clean water to remove the cell debris.

3.5 Analyses

3.5.1 Cell Concentration

The cell concentration was obtained by measuring the optical density of the culture using visible a spectrophotometer (Shimadzu UV-1201). The absorbance of the culture as read at 660 nm. Fresh medium was used as a blank solution. The absorbance values were converted to dry cell weights by the help of the calibration curves shown in Appendix C.

3.5.2 pH Analysis

The pH of the liquid samples taken from the photobioreactor was measured using a standard combination of pH electrode (Mettler Toledo 3311).

3.5.3 Temperature Measurement

Temperature in the indoor experiments was measured using a digital thermometer (Maxi-T) while in the outdoor experiments it was measured using an online thermometer (Elimko Temperature Monitor). The thermometers were inserted in the temperature probe located on top of the photobioreactor.

3.5.4 Light Intensity Measurement

Light intensity measurement was done by a luxmeter (Lutron LX-105 Light Meter). In the indoor experiments, using tungsten lamps, the lux-W/m^2 conversion was determined as 1 W/m^2= 17.5 lux (Uyar, 2008) while in the outdoor experiments under sunlight, it was determined as 1 W/m^2 = 111 lux (Tabanoğlu, 2002).

3.5.5 Gas Composition Measurement

Gas samples were collected using a gas-tight syringe (Hamilton, 22 GA 500μL gas tight No. 1750) from the gas collection column and analyzed by gas chromatography. The gas chromatography equipment (Agilent Technologies 6890N) had a thermal conductivity detector and a Supelco Carboxen 1010 column. In the gas analysis, argon was used as a carrier gas at a flow rate of 26 mL/min the oven, injector and detector temperatures were 140 ºC, 160 ºC and 170 ºC, respectively. A typical gas analysis chromatogram is given in Appendix E.

3.5.6 Organic Acid Analysis

Organic acid analysis was done using HPLC (Shimadzu 10A series) in the Chemical Engineering Department at the Middle East Technical University, Ankara, Turkey. Liquid samples were filtered using a 45μm nylon filters (Millipore, 13 mm) to get rid of impurities. The filtered samples were analyzed by an Alltech IOA-1000 (300 mm x 7.8 mm) HPLC column. In the analysis, 0.085 M H_2SO_4 was used as the mobile phase and the oven temperature was

kept constant at 66 °C. A low gradient pump (Shimadzu LC-10AT) with a degasser (Shimadzu DGU-14A) was used to maintain the mobile phase flow rate at 0.4 ml/min. An autosampler (Shimadzu SIL-10AD) injected 10µl sample into the system and a UV detector (Shimadzu FCV-10AT) with absorbance set at 210 nm was used to determine the component separation. Peak values for the samples were automatically recorded and concentrations determined manually by constructing calibration curves for different concentrations of pure organic acid standards. The organic acids measured were lactic acid, formic acid, acetic acid, propionic acid and butyric acid. Shown in Appendix F is a sample HPLC chromatogram and a calibration curve for acetic acid.

3.5.7 Glutamic Acid Analysis

Glutamic acid analysis was carried out using HPLC (Shimadzu LC 20 A- Prominence Series) in the Chemical Engineering Department at the Middle East Technical University, Ankara, Turkey. Since some amino acids were not visible at UV wavelenghths because of their low absorbance values, derivatization of the glutamic acid was necessary to convert it to a UV recognizable component. Derivatization also helped removed interferences that may be caused by other components in the solution mixture. Pre-column derivatization (PITC (phenyl isothiocyanate) derivatization reaction) was applied in the glutamic acid HPLC analysis. In this process, PITC (acetonitrile diluted solution) and triethylamine (acetonitrile diluted solution) were used to derivatize the photobioreactor liquid sample. The mixture was thourougly mixed and incubated for 20 minutes in an oven set at 40^0C. It was then neutralized using aqueous hydrochloric acid solution after cooling to room temperature. Finally, n-Hexane was used to extract the derivatized sample. The aqueous phase was injected to the HPLC for analysis. A detailed summary of the PITC derivatization process is given in Appendix G.

In the HPLC analysis, two mobile phases were used. Mobile Phase A contained 10 mmol/l KH_2PO_4 and K_2HPO_4 buffer while mobile Phase B was a

50% v/v diluted acetonitrile solution. The mobile phase was pumped at 1.0 ml/min flowrate using a high pressure gradient pump module (Shimadzu AC-20AD) having a degasser (Shimadzu DGU-20A_5). Teknokroma mediterranea Sea 18 (150 mm x 4.6 mm, i.d 3μm) C18 HPLC column was used in the analysis. The column was operated at 40^0C. 10 μl of the derivatized sample was injected automatically by the auto-sampler (Shimadzu SIL-20 ACHT) and a PDA detector (Shimadzu SPD-M20 A) set at absorbance 254 nm was used to detect the peaks. As in the organic analyses, the sample peak values were automatically recorded and the concentrations determined manually by constructing calibration curve for pure glutamic acid standard. Shown in Appendix G is a summary of the HPLC operation conditions for glutamic acid analysis, the composition of the mobile phases and derivatization chemicals, a sample glutamate chromatogram and calibration curve.

3.5.8 Bacteriochlorophyll *a* Measurement

For the determination of bacteriochlorophyll *a* content of the bacteria, 1 ml sample from the photobioreactor was centrifuged at 10000 rpm for 10 minutes. The supernatant was then discarded and acetone-methanol mixture (7:2 v/v) was added for extraction of bacteriochlorophyll *a* (Cohen-Bazire, 1957). The mixture was vortexed for 1 minute to homogenize. Greater than 92% of the bacteriochlorophyll is extracted by this method (Biel, 1986). After vortexing, the mixture was centrifuged at 10000 rpm for 10 minutes to remove the proteins (Hirabayashi, 2006). After 10 minutes, the supernatant was separated and its absorbance was measured at 770 nm (extinction coefficient=76 $mM^{-1}cm^{-1}$) using a spectrophotometer (Shimadzu UV-1201). The acetone-methanol mixture was used as blank (Clayton, 1966). The cellular bacteriochlorophyll *a* content was calculated by considering the cell dry weight. Shown in Appendix H is a sample calculation for the determination of bacteriochlorophyll *a*.

3.6 Biomass Recycle

3.6.1 The Biomass Recycle Procedure

800 ml effluent from the photobioreactor was equally divided (200 ml) into four 250 ml vials in a sterile laminar cabin. The vials were the centrifuged (Sigma- model 4K15) at 4000 rpm and 20^0C for 20 minutes. After centrifuging, the supernatant in the vials were discarded. UNsder aseptic conditions in a sterile laminar cabin, hydrogen production medium is then added to the vials containing the packed cells and the mixture shaken to suspend the cells. The re-suspended cells in the four vials were combined in a calibrated glass bottle and filled to 800 ml using 40 mM/ 2 mM Ac/Glu hydrogen production medium. The 800 ml medium containing the bacteria cells were then fed back into the photobioreactor as decribed in Section 3.4.4.

3.6.2 Determination of the Amount of Biomass Recycle

800 ml effluent from the photobioreactor was equally divided (200 ml) into four 250 ml vials in a sterile laminar cabin. The vials were weighed before the effluents were put in them. The effluents were then centrifuged (Sigma – Model 4K15) at 4000 rpm and 20^0C for 20 minutes. The supernatant was discarded after centrifuging. The pellets, together with the vials were dried for 96 hours in an oven set at 60^0C. Then the vials containing the pellets were weighed again. The amount of biomass recycled was determined by subtracting the weight of the empty vials from the total weight of vials and dried pellets.

3.7 Ammonium Ion Removal

3.7.1 Gördes Clinoptilolite Batch Pre-treatment

Pre-treatment of Gördes clinoptilolite aims to replace the exchangeable cations such as K^{+1}, Na^{+1}, Mg^{+2}, Ca^{+2} in the zeolite with more easily removable ones such as Na^{+} ions. This improves the zeolite's effective exchange capacity, thus a better performance in ion exchange applications.

Clinoptilolite samples obtained from Gördes-Manisa, Turkey, were batch pre-treated (Bayraktaroğlu, 2006). The samples were first ground and then sieved to 200-500 μm particle size. 140 g of the sieved samples were placed in a 500 ml Erlenmeyer flask and 500 ml 1 M NaCl solution added on it. The solution was then mixed continuously for 3 days using a magnetic stirrer. Daily, used 500 ml NaCl solution was replaced with fresh 500 ml 1M NaCl solution. After 3 days, the stirrer was stopped and the zeolite was separated from the solution through decantation. It was washed using deionized water to get rid of NaCl traces. Sodium ion presence was checked using 1 N $AgNO_3$ solution. After washing, the clinoptilolite was dried in an oven set at 75^0C for 12 hours.

3.7.2 Dark Fermenter Effluent Ammonium Ion Removal

The pre-treated clinoptilolite was used to reduce the ammonium ions concentration in a dark fermenter effluent of sugar-beet thick juice molasses. The DFE composition is given in Appendix K1. Batch treatment was applied. 0.6 g of pre-treated clinoptilolite and 50 ml the DFE sample were put in a bottle and mixed using a magnetic stirrer. The experimental setup is shown in Figure 3.5.

Figure 3.5 Ammonium ion removal in the dark fermenter effluent of sugar-beet thick juice molasses using clinoptilolite.

At regular intervals, samples were taken and ammonium ion concentration was measured using an ammonium ion electrode (Russel Model 95-5129). Before each measurement, the ammonium electrode was calibrated using 10^{-4}M - 10^{-1}M NH_4Cl standard solutions. A sample calibration curve is shown in Appendix K2. Prepared 10 mM NH_4Cl solutions were also batch treated.

3.7.3 Total Nitrogen Measurement

The total nitrogen content on the molasses DFE before and after ammonium ion reduction using clinoptilolite was measured. HACH (DR/2400) Total Nitrogen kit with the low measurement range 0-25 mg/l N was used in this experiment. Alkaline persulfate digestion method whereby all forms of nitrogen in the sample were converted to nitrate was applied. Diluted to samples of molasses (1 mg/l) and 10 mg/l and 25 mg/l NH_4Cl standard solutions were digested. The standard samples were digested to check on the accuracy of the measuring kit. 10 ml of the samples were placed in vials and nitrogen persulfate reagent added. The mixture was digested in a COD set at 105^0C for 30 minutes. Then sodium metabisulfite was added to eliminate halogen oxide interferences and then chromotropic acid was added under

strongly acidic conditions to react with nitrate to form a yellow complex with absorbance maximum at 410 nm. The resulting sample was then measured using HACH spectrophotometer that gave the total N reading in mg/l.

In this method, firstly, the sample was digested (N converted to NO_3^-) using nitrogen persulfate reagent pillow. Digestion took place in vials placed in a COD reactor and run for 30 minutes at 103-106^{0}C. After digestion, sodium metabisulfite was added to eliminate halogen oxide interferences, and then chromotropic acid was added and left for 5 minutes for the reaction to go to completion. The chromotropic acid under strongly acidic conditions reacted with nitrate to form a yellow complex with absorbance maximum at 410 nm. The total N amount in the samples was then measured using a HACH spectrophotomer that gave the total N reading in mg/l. The results were read with regards to a blank prepared during the experiment.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Hydrogen Production in Outdoor Continuous Panel Photobioreactors with Different *R. capsulatus* Strains

Hydrogen production experiments using the photosynthetic bacteria *Rhodobacter capsulatus* were carried out in continuous panel photobioreactors in indoor and outdoor conditions. *Rhodobacter capsulatus* (DSM 1710), heat adapted *Rhodobacter capsulatus* (DSM 1710) and *Rhodobacter capsulatus* YO3 (Hup^-) bacterial strains were tested in outdoor conditions, under natural sunlight during September-December, 2008. Defined hydrogen production medium containing the standard basal medium, acetate (40 mM) and glutamate (2 mM) described in Appendix A was used as feed to the 8 l panel photobioreactors. A dilution rate of 0.8 l/ day was appplied. The outdoor experiments showed that *Rhodobacter capsulatus* YO3 (Hup^-) had a high hydrogen production rate in winter, under the low light intensity and temperature, but unstable biomass growth. Hence indoor experiments in 8 l photobioreactors with continuous illumination and controlled temperature were carried out to optimize the feed composition. Different feed compositions containing acetate (40-80 mM) and glutamate (2-4 mM) were examined for stable biomass growth and hydrogen production. In the indoor and outdoor experiments, 40 mM/2 mM Ac/Glu containing hydrogen production media was used at startup because it showed good bacterial growth and hydrogen production in batch experiments with *Rhodobacter capsulatus* (Özgür *et al*., 2009). Since the outdoor photobioreactors were not continuously illuminated, the hydrogen productivities for the outdoor experiments were determined

taking into consideration only the daytime illumination period. The illumination periods were taken as shown in the Table 4.1.

Table 4.1 Outdoor sunlight illumination periods.

Month	Illumination period (hour)
September	12
October	11
November	10
December	8

4.1.1 Continuous Panel Photobioreactor with *R. capsulatus* (DSM 1710)

An 8 l outdoor photobioreactor with *R.capsulatus* DSM (1710) bacterial strain was operated in September, 2008. The PNS bacteria was grown as described in Section 3.4.1 and the photobioreactor started using the procedure stated in Section 3.4.3. 24% bacterial inoculation and hydrogen production medium containing 30 mM acetate and 2 mM glutamate was introduced into the photobioreactor at startup. The system was started in indoor conditions to enable easier growth of the bacteria as the high outdoor temperatures (35-40 ^{0}C) in September would hinder proper growth. In the indoor conditions, illumination (1500 lux~86W/m^2) was provided by four 60W tungsten lamps and the room temperature was controlled by an air conditioner. After inoculation, the photobioreactor was operated in batch mode (Phase I) with no media feeding until the bacteria reached stationary phase at 1 gdcw/l$_c$ on the 4th day. Biomass grew at 0.019 h^{-1}. Then the system was moved to outdoor conditions and continuous feeding was introduced. The photobioreactor was continuously fed using the hydrogen production medium containing acetate (40 mM) and glutamate (2 mM) and a dilution rate of 800 ml (10% v/v) for 17 days. Shown in Figure 4.1 is the biomass growth and cumulative hydrogen

production for the experiment. Stable biomass growth was obtained at 1 gdcw/l_c in Phase II. The hydrogen production rate at the startup was high (2.2 l/day) due to continuous illumination and controlled temperature (30-32^{0}C). After moving to the outdoor conditions and beginning of continuous feeding on the 4th day, the highest hydrogen production rate decreased to 0.4 l/day. This was due to the slight decrease in temperature and light intensities in outdoor conditions as shown in Figures 4.2 and 4.3 respectively. Hydrogen production levels have been shown to vary with changing light and temperatures in outdoor conditions (Wakayama *et al*., 1998; Özgür *et al*., 2009). In the outdoor conditions, high daytime temperatures averaging 35^{0}C and low temperatures at night (10^{0}C) were experienced in the month of September, 2008 as shown in Figure 4.2. The daily sunlight intensities in Ankara ranged 1.5-5.5 kwh/m^2.day during this period as shown in Figure 4.3.

However, on the 9th day, the hydrogen production rate increased to 1.1 l/day. The increase can be attributed to the bacteria adapting to the outdoor conditions and the light/dark cycle. Light/dark cycle has been stated to stimulate hydrogen production (Koku *et al*., 2003; Uyar *et al*., 2007). The productivity dropped again at the end of the month with decreasing light intensities and temperatures as seen in Figures 4.2 and 4.3. The average hydrogen productivities at the startup (Phase I) and in continuous operation (Phase II) were 320 ml H_2/l_c.day and 92 ml H_2/l_c.day respectively. Gas chromatography results showed that the total gas produced was 90-95% hydrogen with the rest being carbondioxide.

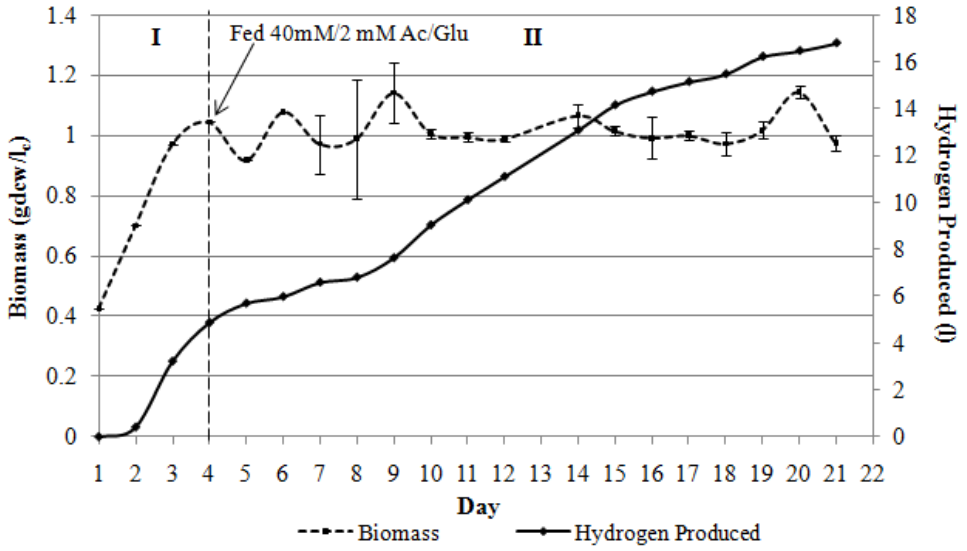


Figure 4.1 The biomass growth and hydrogen production for *R. capsulatus* (DSM 1710) CPBR experiment (13th September, 2008 – 3rd October, 2008).

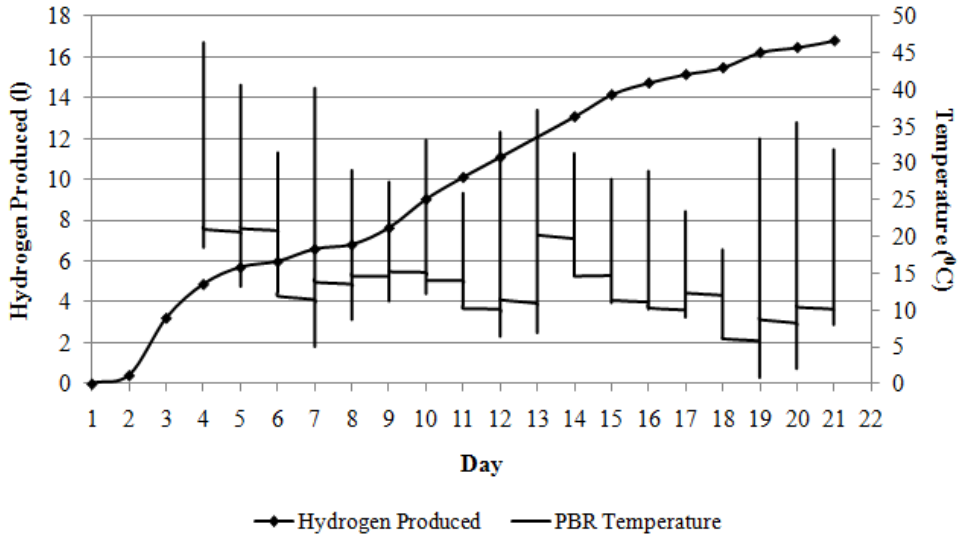


Figure 4.2 The photobioreactor temperature variation and cumulative hydrogen production in the *R. capsulatus* (DSM 1710) CPBR experiment (13th September, 2008 – 3rd October, 2008).

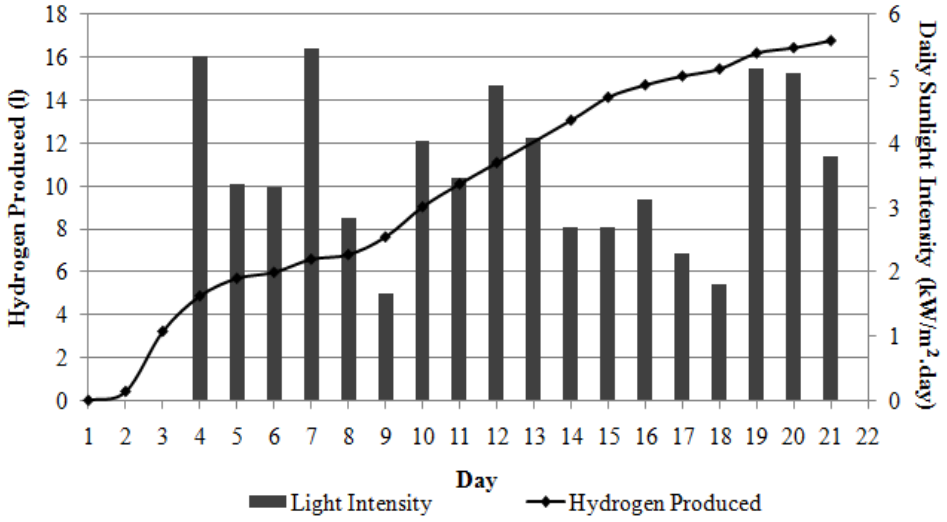


Figure 4.3 Daily average sunlight intensity and cumulative hydrogen production in the *R. capsulatus* (DSM 1710) CPBR experiment (13th September, 2008 – 3rd October, 2008). Sunlight intensity data was obtained from the National Meteorology Institute of Turkey.

The pH of the photobioreactor ranged between 7.4 and 7.6, which is in the optimum range for hydrogen production (Sasikala *et al*., 1993, Arik *et al*., 1996). The pH change is shown in Figure 4.4.

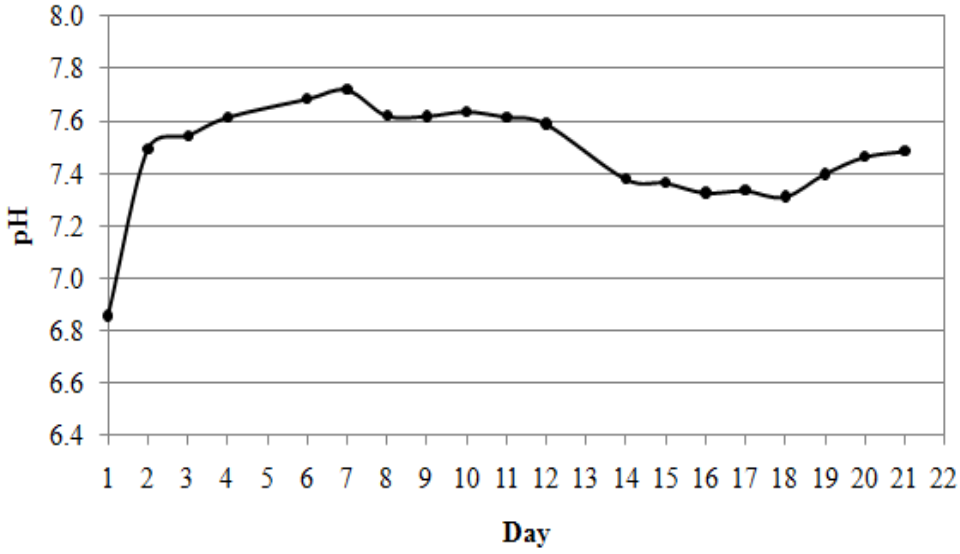


Figure 4.4 The change in pH in the *R. capsulatus* (DSM 1710) CPBR experiment (13th September, 2008 – 3rd October, 2008).

Shown in Figure 4.5 is the organic acid utilization in the *R. capsulatus* (DSM 1710) photobioreactor. It is observed that acetic acid was steadily consumed at the startup in Phase I as the bacteria grew. A substrate conversion efficiency of 35% and acetate conversion of 74% were obtained in this phase. In Phase II, after the introduction of continuous feeding, acetic acid concentration averaged 3 mM. The acetate conversion increased to 87% as bacteria utilized most of the acetic acid for hydrogen production and survival. 28% substrate conversion efficiency was obtained. Also, it is observed that there was a slight increase in acetic acid after the 15th day. This could be due to the decrease in temperatures and light intensities at the end of the month as fall approached as seen in Figures 4.2 and 4.3. Lactic acid, formic acid, propionic acid and butyric acid were formed in very low amounts. Summarised in Table 4.2 is the results of the outdoor panel photobioreactor using *R. capsulatus* (DSM 1710).

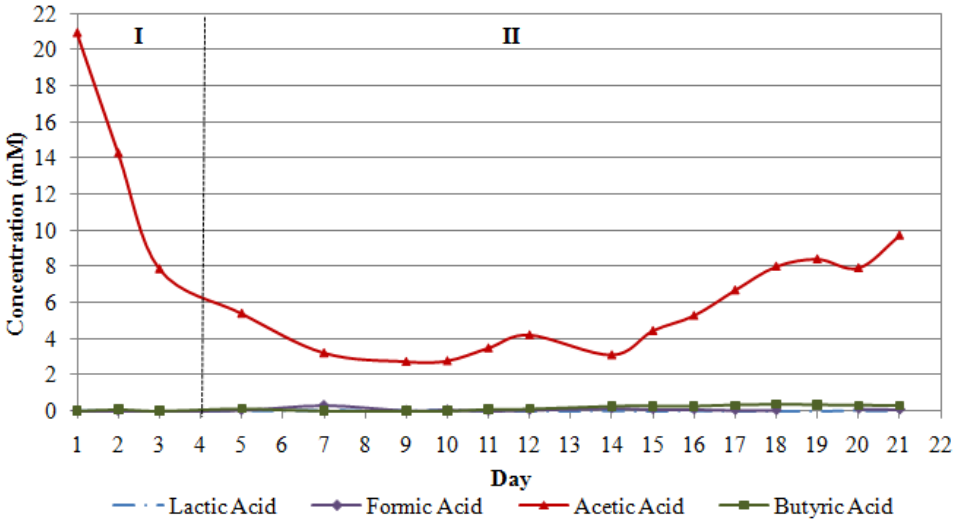


Figure 4.5 Organic acid utilization in the *R. capsulatus* (DSM 1710) CPBR experiment (13th September, 2008 – 3rd October, 2008).

4.1.2 Continuous Panel Photobioreactor with Heat Adapted *R. capsulatus* (DSM 1710)

An outdoor experiment using heat adapted *R. capsulatus* (DSM 1710) was carried out in a continuous 8 l panel photobioreactor. The panel photobioreactor was started in outdoor conditions with 14% bacterial inoculation and hydrogen production medium containing 40 mM / 2 mM Ac/Glu. Continuous feeding was started on the 7th day when the biomass reached 0.94 gdcw/l_c. A daily 800 ml (10%) hydrogen production medium containing acetate (40 mM) /glutamate (2 mM) was fed continuously. This experiment was carried out between September-December, 2008.

Shown in Figure 4.6 is the biomass growth and cumulative hydrogen production of the heat adapted bacteria. Steady biomass growth at a rate of 0.012 h^{-1} is observed in Phase I. The cell dry weight fluctuated between 1.1-0.75 gdcw/l_c in Phase II after the start of continuous feeding. Temperatures ranged between 15-30^{0}C in this phase as shown in Figure 4.7. In Phase III, due to the onset of winter on the 53rd day, the PBR was moved to a glasshouse to

prevent the bacteria from freezing. Electric heaters were used at night to maintain temperatures above 0^0C as described in Section 3.3.3. During this period the sunlight intensity ranged between 0.5-3 kwh/m^2.day as seen in Figure 4.8. Biomass stabilized at around 0.75 gdcw/l_c and eventually decreased to 0.45 gdcw/l_c . The maximum hydrogen production rate observed at startup (Phase I) was 1.34 l/day but it decreased to 0.60 l/day in Phase II with continuous feeding. The production rate further decreased to 0.45 l/day in Phase III. This decrease is attributed to the decrease in temperature and light intensities in the outdoor conditions as shown in Figures 4.7 and 4.8 respectively. Average hydrogen productivities of 60, 72 and 40 ml H_2/l_c.day were obtained inPhases I, II and III respectively. Gas chromatography results showed that the total gas produced in this experiment was 90% hydrogen with the rest being carbondioxide. The pH of the culture averaged 7.4 as shown in Figure 4.9.

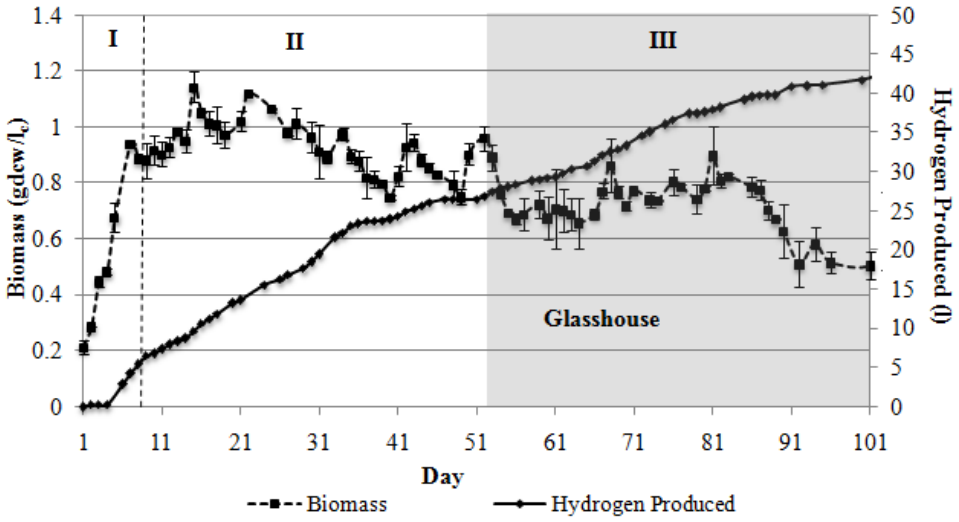


Figure 4.6 The biomass growth and hydrogen production for the heat adapted *R. capsulatus* (DSM 1710) CPBR experiment (20th September, 2008 - 29th December, 2008).

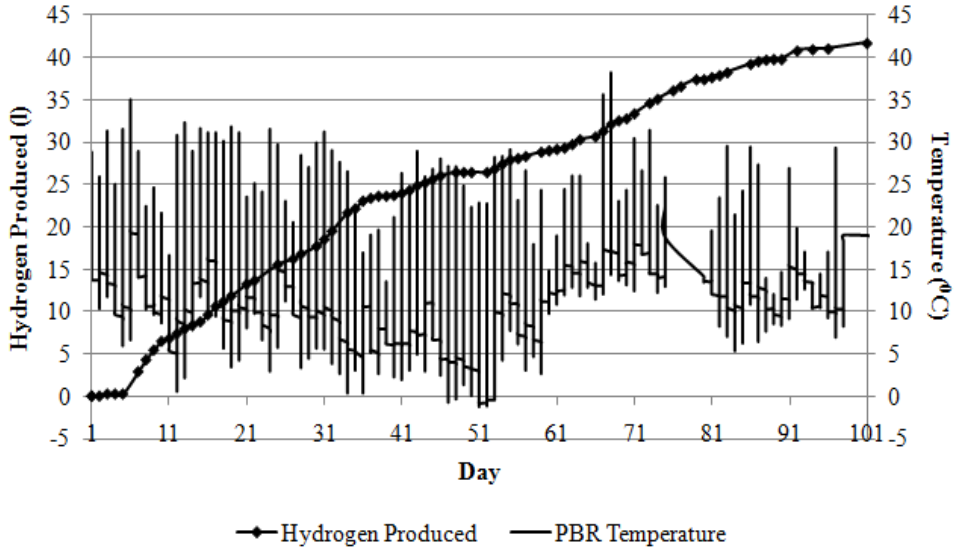


Figure 4.7 The photobioreactor and air temperature variation in the heat adapted *R. capsulatus* (DSM 1710) CPBR experiment (20th September, 2008 - 29th December, 2008).

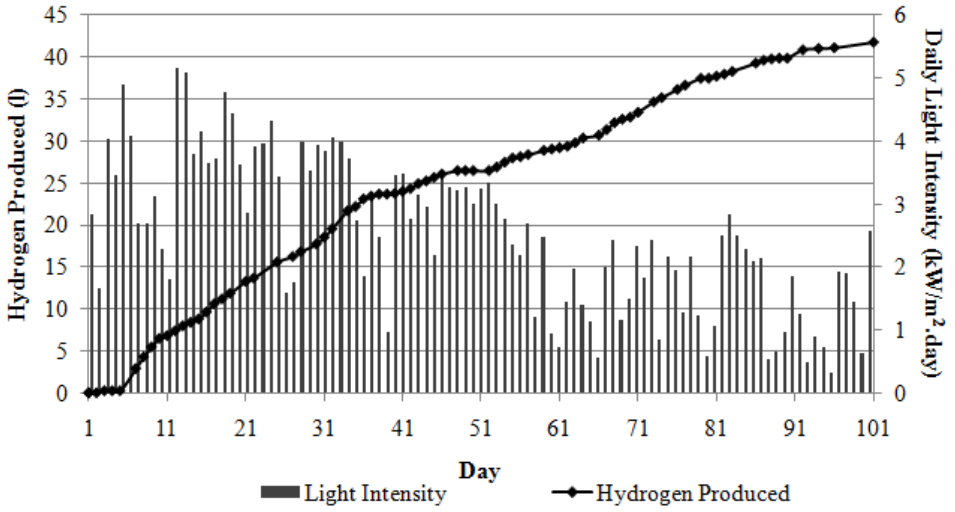


Figure 4.8 Daily average sunlight intensity and cumulative hydrogen production in the heat adapted *R. capsulatus* (DSM 1710) CPBR experiment (20th September, 2008 - 29th December, 2008). Sunlight intensity data was obtained from the National Meteorology Institute of Turkey.

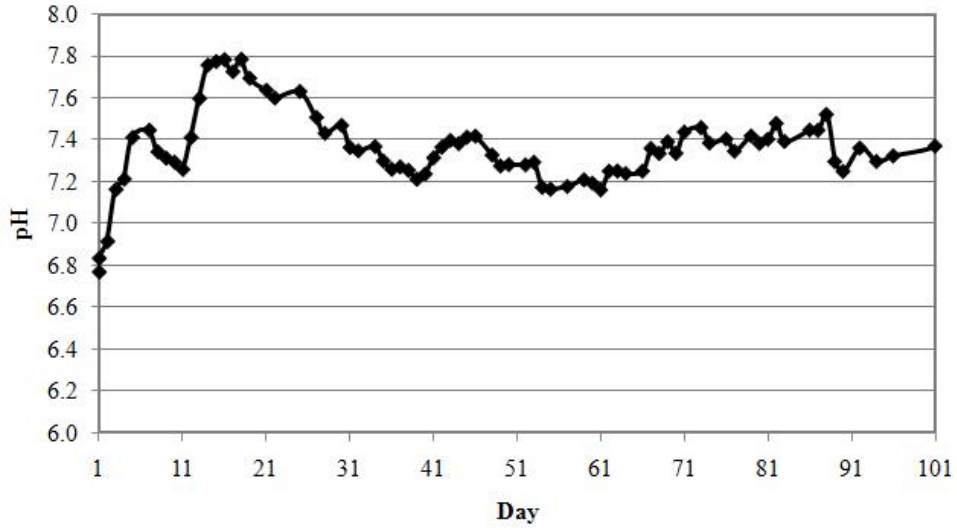


Figure 4.9 The change in pH in the heat adapted *R. capsulatus* (DSM 1710) CPBR experiment (20th September, 2008 - 29th December, 2008).

Shown in Figure 4.9 is the organic acid utilization in the heat adpated *R. capsulatus* PBR. In Phase I, acetic acid fed at the startup was steadily consumed as the bacteria grew. An acetate conversion of 72% and substrate conversion efficiency of 23% was obtained in this phase. In Phase II, after the introduction of continuous feeding, acetic acid concentration varied between 6-10 mM for 31 days. A 79% acetate conversion and 20% substrate conversion efficiency was obtained in this Phase II (calculations were done for the 31 days when acetate consumption was stable). In Phase III, acetic accumulation is observed. This could be attributed to the low light intensity during this period as seen in Figure 4.8. Lactic acid, formic acid, propionic acid and butyric acid were formed in low amounts as seen in Figure 4.10. Summarised in Table 4.2 is the results of the outdoor panel photobioreactor using heat adapted *R. capsulatus* (DSM 1710).

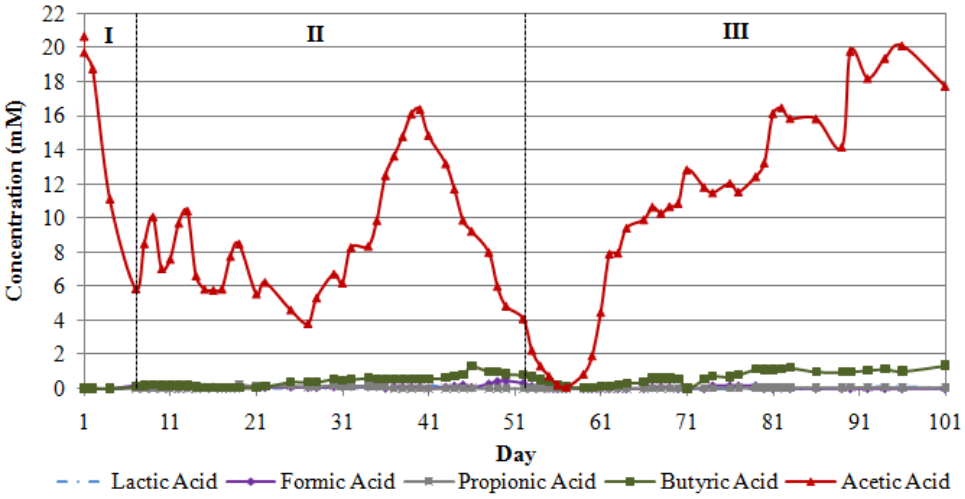


Figure 4.10 Organic acid utilization in the heat adapted *R. capsulatus* (DSM 1710) outdoor experiment CPBR experiment (20th September, 2008 - 29th December, 2008).

4.1.3 Continuous Panel Photobioreactor with *R. capsulatus* YO3 (Hup$^-$)

This experiment was carried out between October-December 2008. An 8 l panel photobioreactor was setup in outdoor conditions using *R. capsulatus* YO3 (Hup$^-$). The PNS bacteria was grown as described in Section 3.4.1 and the photobioreactor started using the procedure stated in Section 3.4.3 in outdoor conditions. 24% bacterial inoculation and hydrogen production medium containing acetate (40mM) /glutamate (2mM) was used at startup. Shown in Figure 4.11 is the biomass growth and the cumulative hydrogen production.

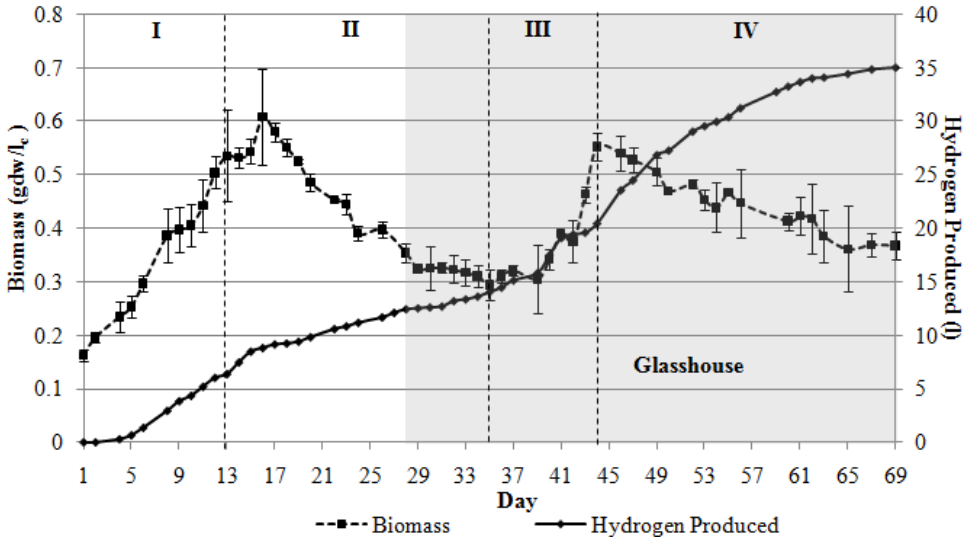


Figure 4.11 The biomass growth and hydrogen production for *R. capsulatus* YO3 (Hup$^-$) experiment CPBR experiment (16th October – 24th December, 2008).

In Phase I, slow bacterial growth was observed. This was because of the varying temperature and light intensity in the outdoor conditions. The photobioreactor temperature ranged between 20-35 ^{0}C during the day and it was about 5^{0}C at night as seen in Figure 4.12. The sunlight intensity was 1-4 kwh/m^2.day in this phase as seen in Figure 4.13. Average hydrogen production rate of 66 ml H_2/l$_c$.day was obtained in this Phase I.

In Phase II, continuous feeding of the hydrogen production medium containing 40 mM/2 mM Ac/Glu commenced on the 13th day after the bacteria had grown to 0.535 gdcw/l$_c$. Biomass decreased from 0.6 to 0.3 gdcw/l$_c$ in Phase II. Average hydrogen productivity of 50 ml H_2/l$_c$.day was obtained as sunlight intensity ranged between 1-3.5 kwh/m^2.day. On the 27th day (the onset of winter), the PBR was moved to glasshouse to prevent the bacteria from freezing. Electric heaters were used at night to maintain temperatures above 0^{0}C as described in Section 3.3.3. To prevent further decrease in the biomass, the glutamate amount in the feed was increased from 2 mM to 10 mM on the

34th day. This led to the increase in biomass as seen in Phase III. Average hydrogen production rate also increased to 86 ml H_2/l_c.day. After 10 days, on the 45th day, when the biomass concentration had reached the 0.552 gdcw/l_c, the glutamate amount in the feed was again reduced to 2mM. Biomass decrease was again observed in Phase IV. Average hydrogen production rate was 72 ml H_2/l_c.day. Comparing the biomass decrease trends and change in feed composition, it is deduced that the major factor that affected the biomass was the feed composition. The 40 mM/2 mM Ac/Glu hydrogen production medium was insufficient for maintaining stable biomass and hydrogen productivity. Increasing glutamate to 10 mM was beneficial for growth but the amount for stable biomass required optimization. During the experiment, total gas produced consisted 92% hydrogen and the remaining was carbondioxide. The pH ranged between 6.8 to 7 as shown in Figure 4.14.

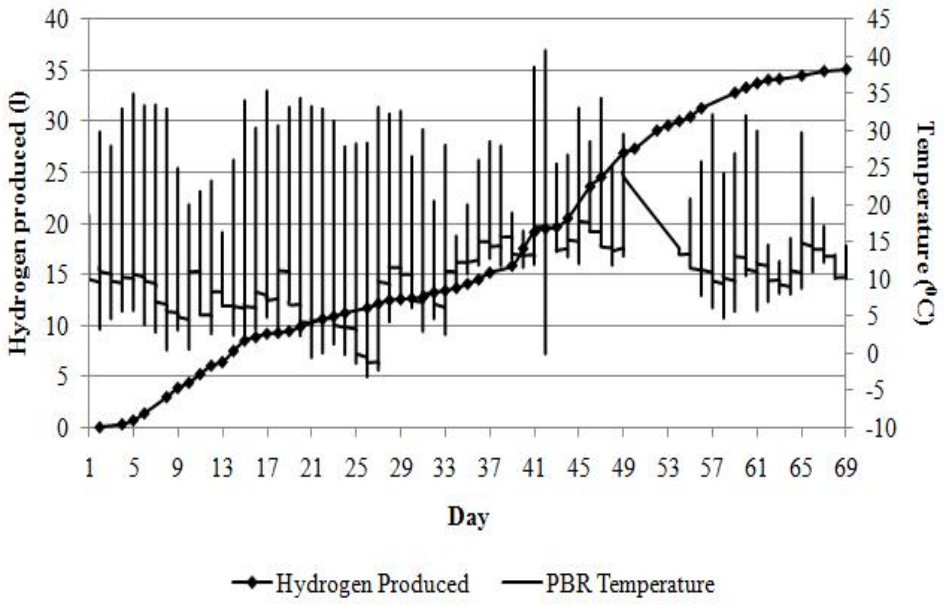


Figure 4.12 The photobioreactor and air temperature variation in the *R. capsulatus* YO3 (Hup$^-$) experiment (16th October – 24th December, 2008).

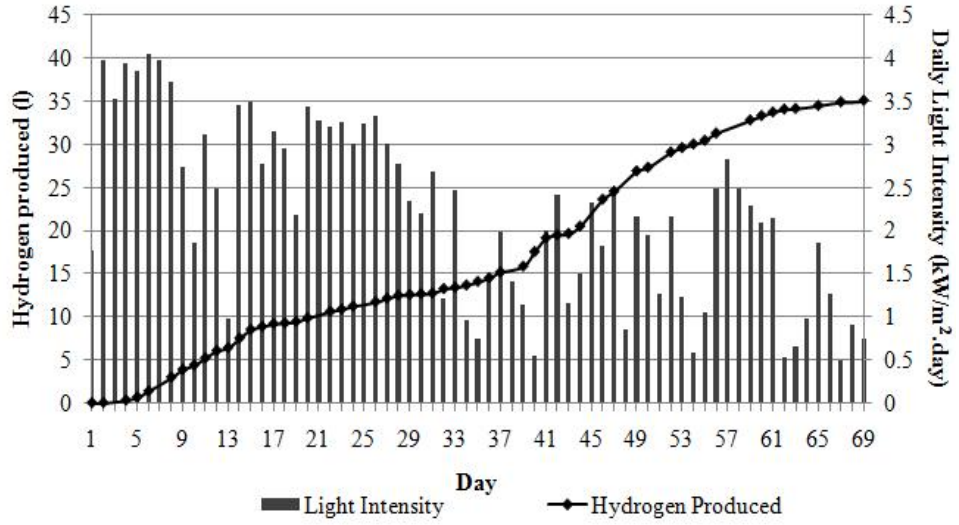


Figure 4.13 Daily average sunlight intensity and cumulative hydrogen production in the *R. capsulatus* YO3 (Hup^-) CPBR experiment (16th October, 2008 - 24th December, 2008). Sunlight intensity data was obtained from the National Meteorology Institute of Turkey.

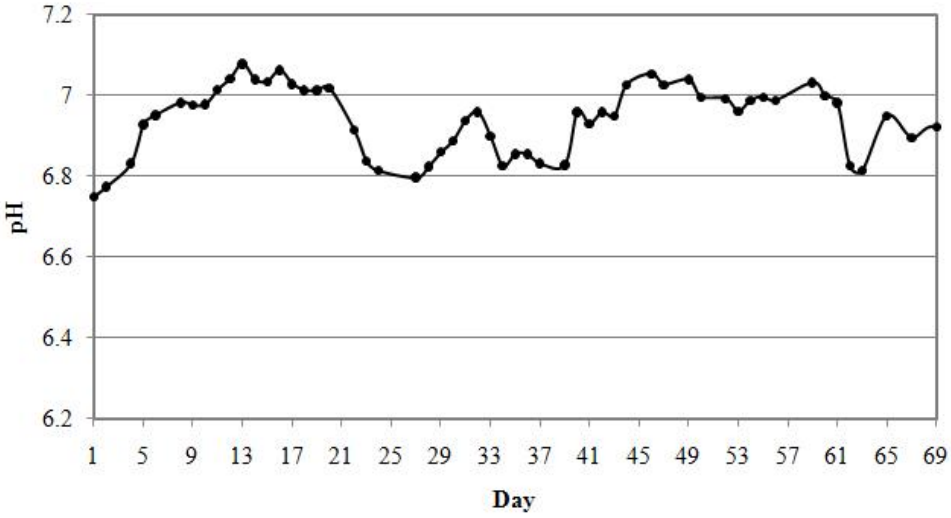


Figure 4.14 The change in pH in the *R. capsulatus* YO3 (Hup^-) outdoor experiment (16th October – 24th December, 2008).

The organic acid HPLC of the *R. capsulatus* YO3 (Hup^-) outdoor experiment is shown in Figure 4.15. It is observed that acetic acid was steadily consumed in Phase I as the bacteria grew. The acetate conversion was 85% with a substrate conversion efficiency of 45 %. In Phase II, most of the acetate fed was utilized and was insufficient to maintain biomass and high hydrogen production. 97 % acetate conversion and 13% substrate conversion efficiency was obtained in this phase. In Phase III, with the increase in glutamate amount and biomass, acetate concentration in the culture increased. This is because the bacteria utilized acetate and the extra glutamate provided for growth and hydrogen production. 79% acetate conversion and 18% substrate conversion efficiency was obtained. In Phase IV, accumulation of acetic acid in the culture was observed. The temperature in this phase ranged between 30-10^{0}C as seen in Figure 4.12. However, low light intensity, 0.5-2.8 kwh/m^2.day was experienced in winter in Ankara as seen in Figure 4.14. This led to the decrease in acetate consumption by the photosynthetic bacteria. 61% acetate conversion and 36% substrate conversion efficiency was obtained in Phase IV. Furthermore, in the the HPLC analysis results, lactic acid, propionic acid and butyric acid were formed in very low concentrations, however, formation of formic acid was observed. On average, 2 mM of formic acid was produced in Phases I, II and III while 6 mM was produced in phase IV. The increase in formic acid could be attributed to the change in mechanism as acetate was not sufficiently utilized by the bacteria because of low light intensity in winter. Tabanöğlu (2002) observed formic acid formation in an outdoor experiment using *R. sphaeroides*. Insufficient lighting of the photobioreactor resulted to anaerobic dark fermentation and formic acid production. A summary of the outdoor experiment done using the *R. capsulatus* YO3 (Hup^-) is shown in Table 4.1.

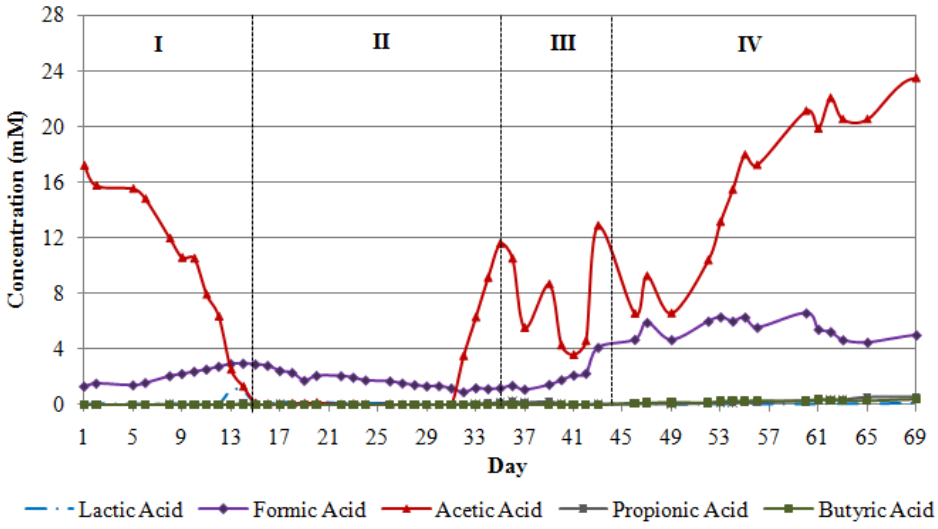


Figure 4.15 Organic acid utilization in the *R. capsulatus* YO3 (Hup$^-$) outdoor experiment (16th October – 24th December, 2008).

4.2 Comparison of the Outdoor Experiments Results

The outdoor continuous panel photobioreactors operated using the three *R. capsulatus* strains were compared with respect to hydrogen productivity, substrate conversion efficiency and acetate conversion. The *R. capsulatus* (DSM 1710) photobioreactor was operated between 13th September – 3rd October, 2008, the heat adapted *R. capsulatus* (DSM 1710) between 20th September – 29th December, 2008 and the *R. capsulatus* YO3 (Hup$^-$) between 16th October – 24th December, 2008.

4.2.1 Hydrogen Productivity

The highest molar hydrogen productivities of the continuous outdoor photobioreactors were plotted in a bar graph as shown in Figure 4.16. A sample calculation is shown in the Appendix I1.2. It was observed that the *R. capsulatus* (DSM 1710), heat adapted *R. capsulatus* (DSM 1710) and *R. capsulatus* YO3 (Hup$^-$) had similar hydrogen productivities of 0.3, 0.3 and 0.4 mmol $H_2/l_c.h$ respectively. The R. capsulatus YO3 (Hup$^-$) strain had a

slightly higher productivity than the *R. capsulatus* (DSM 1710) despite being operated during the winter, under lower temperatures and light intensity. The *R. capsulatus* (DSM 1710) was operated in late summer. Wakayama et al., 1998 showed that hydrogen production levels depended on irradiation of sunlight intensity in outdoor conditions. They obtained maximum hydrogen production rate of 4 $l/m^2.h$ with over 2% light conversion efficiency in outdoor experiments carried out under natural sunlight using *R. sphaeroides* RV. The results obtained in this study nullify the effect of the absence of the uptake hydrogenase enzyme. Özgür et al., 2009 found that *R. capsulatus* YO3 (Hup^-) had a higher productivity than the *R. capsulatus* (DSM 1710) in batch experiments carried out during summer and fall of 2007.

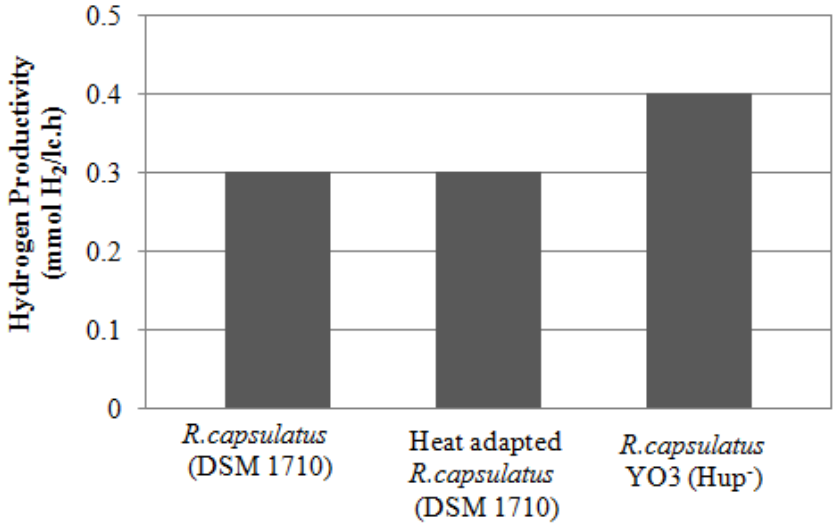


Figure 4.16 Comparison of the outdoor photobioreactors hydrogen productivities.

4.2.2 Substrate Conversion Efficiency (Yield)

The substrate conversion efficiency (yield) was determined as the ratio of the amount of hydrogen produced to the total theoretical hydrogen that can be produced from acetate and glutamate. The highest substrate conversion efficiencies corresponding to the highest hydrogen production values were compared. A sample calculation is shown in the Appendix I2. Shown in Figure 4.17 is the substrate conversion efficiencies comparison. It was observed that despite being operated in winter under lower temperature and light intensity, the *R. capsulatus* YO3 (Hup^-) had the highest substrate conversion efficiency (36 %). The *R. capsulatus* (DSM 1710) strain and its heat adapted version had 28% and 30% substrate conversion efficiencies respectively. Studies done on *R. capsulatus* indicate that the substrate conversion efficiencies vary between 6-72% (Koku et al., 2003). It was reported that the *R. capsulatus* YO3 (Hup^-) strain exhibited higher substrate conversion efficiency (33%) than *R. capsulatus* (DSM 1710) (19%) (Özgür et al., 2009).

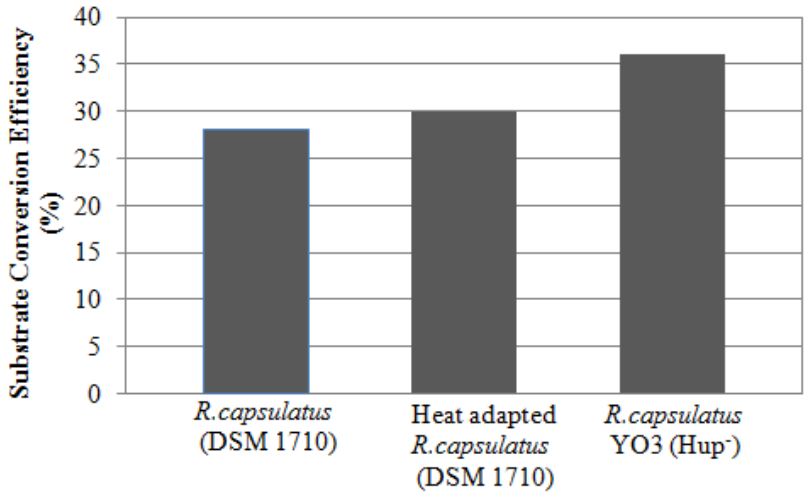


Figure 4.17 Comparison of the outdoor photobioreactors substrate conversion efficiencies.

4.2.3 Acetate Conversion

The acetate conversion, which is the ratio of the acetate utilized to the amount fed, is calclulated as 87% for *R. capsulatus* (DSM 1710), 75% for the heat adapted strain and 61% for *R. capsulatus* YO3 (Hup^-) containing CPBRs. The lower acetate conversion can be attributed to the decreasing biomass concentration and low winter light intensities (2-3 kwh/m^2.day). Low light intensity has been shown to lead to a decrease in the total volume of hydrogen produced (Miyake and Kawamura, 1987; Nakada *et al*., 1995; Yamada *et al*., 1998; Uyar *et al*., 2007). Studies done by Barbosa *et al*. (2001) showed that low light intensities led to lower acetate consumption by PNS bacteria. Using two tungsten lamps, they investigated the effect of light intensity between 40 and 600 μmol photons m^{-2} s^{-1} and found that the highest volume (25 ml H_2 /l.h) was obtained in *Rhodopseudomonas* sp. with light intensity of 680 μmol photons/ m^2.s. Moreover, the high acetate conversion values for *R. capsulatus* (DSM 1710) in outdoor conditions assert the suitability of acetate as a substrate for photosynthetic bacteria (Barbosa *et al*., 2001; Asada *et al*., 2008). Barbosa *et al*. (2001) compared the hydrogen production of three bacterial strains (*Rhodopseudomonas* sp., *Rhodopseudomonas palustris* and a non-identified strain) on four different organic acids (lactate, malate, acetate and butyrate). The substrate conversion conversion yield (72.8%), hydrogen evolved (269 ml H_2 per vessel), hydrogen production rate (25 ml H_2 /l.h^1) and light efficiency (0.9%) were achieved by *Rhodopseudomonas* sp. with acetate as the carbon source. Asada *et al*. (2008) tested 5 strains of agar immobilized PNS bacteria and observed that *R. sphaeroides* IL 106 gave the highest yield of 3.03 mol of hydrogen per acetate when acetate concentration was low. Shown in Figure 4.18 is the acetate conversion comparison.

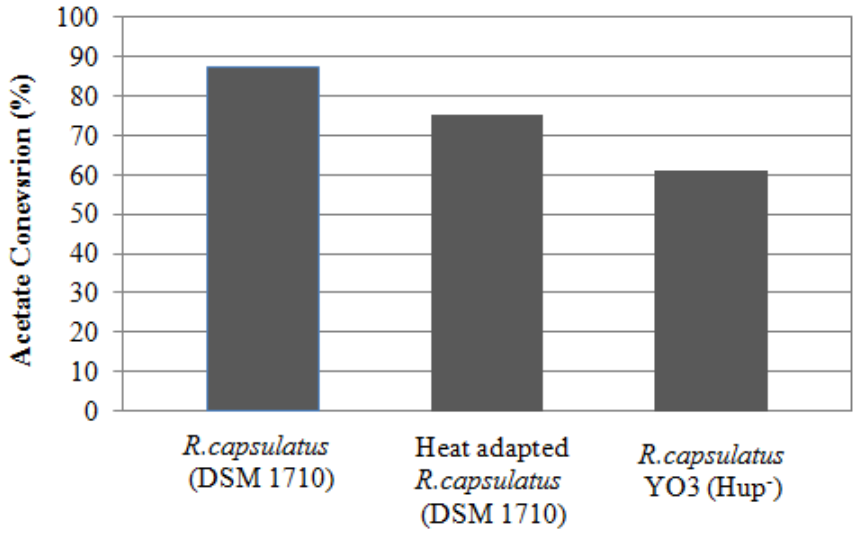


Figure 4.18 Comparison of the outdoor photobioreactors acetate conversions.

Overall, the three bacterial (*R.capsulatus* wildtype (DSM 1710), heat adapted *R. capsulatus* wildtype (DSM 1710) and *R. capsulatus* wildtype YO3 (Hup^-) strains had similar molar hydrogen productivities and were all suitable for long term hydrogen production using acetate as a carbon source. These results were obtained using defined medium described in Appendix A, however, the performance of these strains on the real dark fermenter effluents from thermophilic fermentation remains to be investigated. Theoretically, the mutant (Hup^-) can produce higher hydrogen than its parent (wildtype) strain because of lack of the uptake hydrogenase enzyme. In the outdoor experiments, it was noted that the 40 mM/2 mM Ac feed composition was insufficient to maintain a stable cell concentration and so further studies were foccussed on optimising the feed media composition for the *R. capsulatus* YO3(Hup^-) strain.

Table 4.2 Summary results of the outdoor continuous panel photobioreactors with Different *R. capsulatus* Strains.

Microorganism	Phase	No. of Days	Temperature (°C)		Biomass (g_{dcw}/l_c)	Hydrogen Productivity		Acetate Conversion (%)	Substrate Conversion Efficiency (%)
			Minimum	Maximum		ml. H_2/l_c.day	mmol. H_2/l_c.h*		
R. capsulatus (DSM 1710)	I[a,b] (Indoor)	4	20 ± 4	34 ± 2	1.02	320	0.6	74	35
	II (Outdoor)	17	10 ± 5	32 ± 7	1.00 ± 0.06	92	0.3	87	28
Heat adapted *R. capsulatus* (DSM 1710)	I[a]	7	10 ± 3	29 ± 3	1.02	60	0.2	72	23
	II	46	7 ± 3	28 ± 4	0.92 ± 0.09	72	0.3	79	29
	III	50	9 ± 4	22 ± 7	0.71 ± 0.09	40	0.2	53	18
R. capsulatus YO3 (hup$^-$)	I[a]	13	5 ± 3	26 ± 6	0.535	65	0.3	85	45
	II	21	4 ± 4	28 ± 8	0.44 ± 0.10	50	0.2	97	13
	III	10	13 ± 1	28 ± 7	0.37 ± 0.09	86	0.4	79	18
	IV	25	8 ± 4	23 ± 7	0.44 ± 0.06	72	0.4	61	36

a) Startup period b) Indoor

*A conversion factor of 0.0445 mmol/ml was used in determining the molar hydrogen productivity.

4.3 Effect of Feed Composition on Long Term Hydrogen Production

Feed media optimization experiments to achieve stable biomass growth and high hydrogen production using the *R. capsulatus* YO3 (Hup^-) were carried out in indoor conditions, under continuous illumination and controlled temperature.

Experiments with biomass recycling to prevent the biomass decrease was carried out. Also, experiments in which the C/N ratio was kept constant at 45 as in the 40 mM/2mM Ac/Glu feed, were carried out. The results of the 40 mM/2 mM/Ac/Glu feed with or without biomass recycle, 60 mM/3 mM Ac/Glu feed and 80 mM/4 mM Ac/Glu feed indoor experiments are given.

4.3.1 Feed Composition: 40 mM Acetate and 2 mM Glutamate

An indoor experiment was carried out in a continuous 8 l panel photobioreactor using *R. capsulatus* YO3 (Hup^-) strain between September-November, 2008. The PNS bacteria was grown as described in Section 3.4.1 and the photobioreactor started in indoor conditions using the procedure stated in Section 3.4.3. 23% bacterial inoculation and hydrogen production medium containing acetate (40 mM) and glutamate (2 mM) were used at startup. In continuous operation, the photobioreactor had a daily dilution rate of 10% of the reactor volume; 800 ml of the 40 mM/2 mM Ac/Glu hydrogen production medium was fed every day. An air conditioner was used to control the room temperature and constant illumination (1500 lux~86W/m^2) was provided by four 60W tungsten lamps. Figure 4.19 illustrates the biomass and hydrogen production results of the indoor experiment.

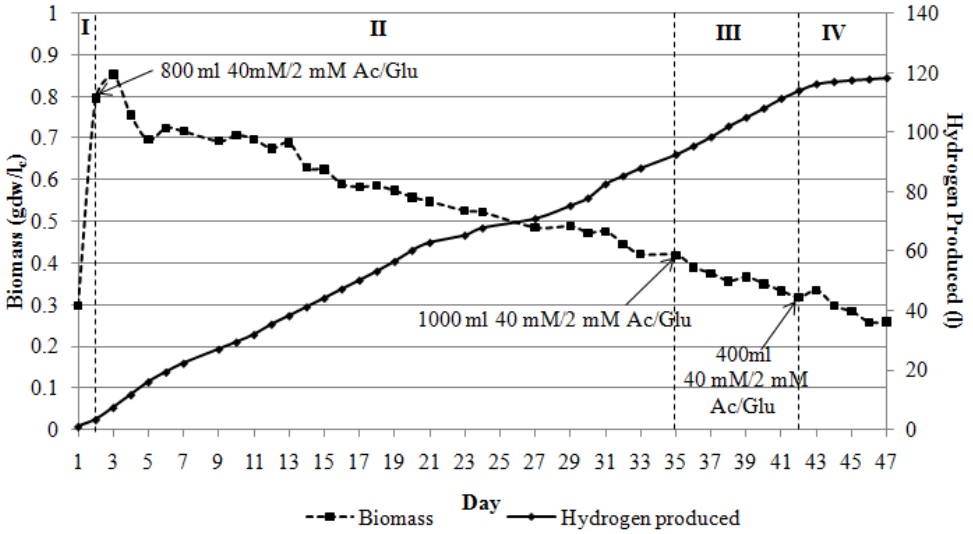


Figure 4.19 The biomass growth and cumulative hydrogen production in the 40 mM/2 mM Ac/Glu fed CPBR (18th September, 2008 - 2nd November, 2008).

In Phase I, because of controlled temperature and continuous illumination provided in the indoor conditions, a fast biomass growth (μ=0.061 h^{-1}) was observed. Biomass concentration of 0.8 gdcw/l_c and hydrogen production rate of 9 ml H_2/l.h was obtained in this phase. Continuous feeding was started on the 2nd day of the experiment. As seen in Phase II, there was continuous decrease in biomass after the start of continuous feeding. The biomass concentration decreased to 0.42 gdcw/ l_c after 31 days. On the contrary, there was high stable hydrogen production rate of 15 ml H_2/l.h in this phase. Since the temperature and light intensity were controlled, the feed composition being insufficient was the reason for the biomass decrease. It was suggested to increase the dilution rate slightly from 800 ml feed medium to 1000 ml per day. As seen in Phase III, the biomass further decreased to 0.33 gdcw/l_c. Hydrogen production rate (16 ml H_2/l.h) similar to Phase II was obtained. In Phase IV, the dilution rate was halved to 400 ml feed medium per day. Hydrogen production stopped in this phase because the daily 400 ml feed medium given was not enough for biomass growth and hydrogen production.

Another reason was that the biomass concentration (0.25 gdcw/ l_c), had dropped below the 0.30 gdcw/l_c critical hydrogen production (threshold) value (Eroğlu *et al.*, 2008). Average pH value of 7.1 and temperature of 32^0C were obtained in the experiment as shown in Figure 4.20.

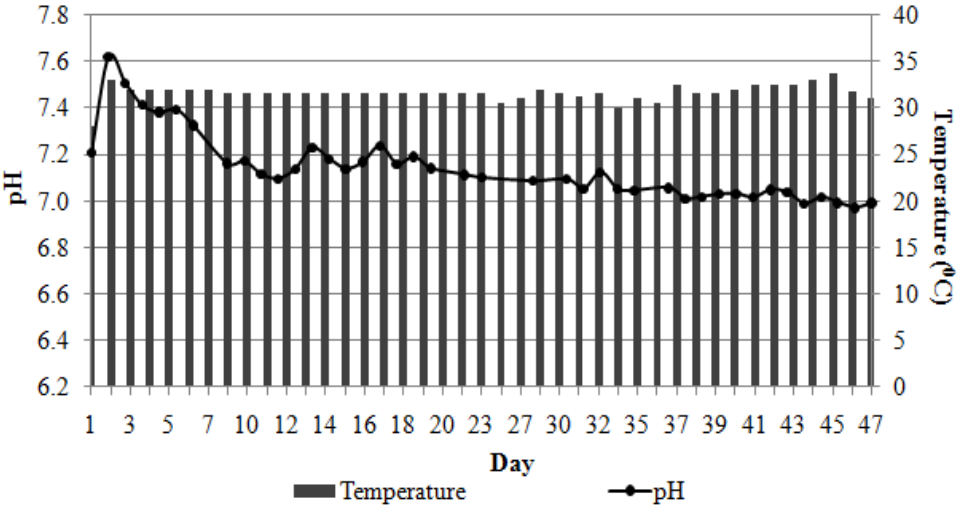


Figure 4.20 The photobioreactor temperature and pH change in the 40 mM/2 mM Ac/Glu fed CPBR experiment (18th September, 2008 - 2nd November, 2008).

The HPLC analysis results are illustrated in Figure 4.21. It is observed that acetic acid was rapidly consumed by the bacteria in Phase I. Acetate conversion (82%), substrate conversion efficiency (20%) and light conversion efficiency (0.63%) was obtained. The high acetate conversion and low substrate conversion efficiency portrays that most of the acetate was used for growth. In Phase II, with the introduction of continuous feeding, the acetate concentration in the system remained low; ranging between 1 mM to 2 mM. This range increased to 3 mM at the end of Phase II as less acetate was consumed due to the decreasing biomass. 99% acetate conversion, 96%

substrate conversion efficiency and 0.84% light conversion efficiency was obtained in this phase. This corresponds to the high 15 ml H_2/l_c.h hydrogen productivity in this phase. In Phase III, the acetate conversion and substrate conversion efficiency slightly decreased to 90% and 91%. The decrease had little effect on hydrogen productivity as it remained at 15 ml H_2/l_c.h. Average acetate concentration in this phase was 4 mM. In Phase IV, as seen, acetate was completely consumed. 100% acetate conversion, 19% substrate conversion efficiency and 0.35% light conversion efficiency was obtained. The low substrate conversion efficiency shows that the bacteria utilized the given acetic acid for biomass formation (maintenance purposes) instead of hydrogen production. Also from the HPLC analysis results, it was found that lactic acid and butyric acids were formed in very low concentrations while formic acid accumulated in the system. On average, 6 mM formic acid concentration was formed in the photobioreactor. The formation of formic acid in the system could be attributed to low light intensity. Formate is a fermentation end product. Studies on the *Rhodobacter* species have shown that the bacteria can switch to fermentative mode and produce organic acids if the illumination is below a threshold value. It shifts back its metabolism to photofermention in the presence of sufficient light (Eroğlu *et al*., 2008). Shown in Table 4.3 is a summary of the 40 mM/2 mM Ac/Glu continuous panel photobioreactor experimental results.

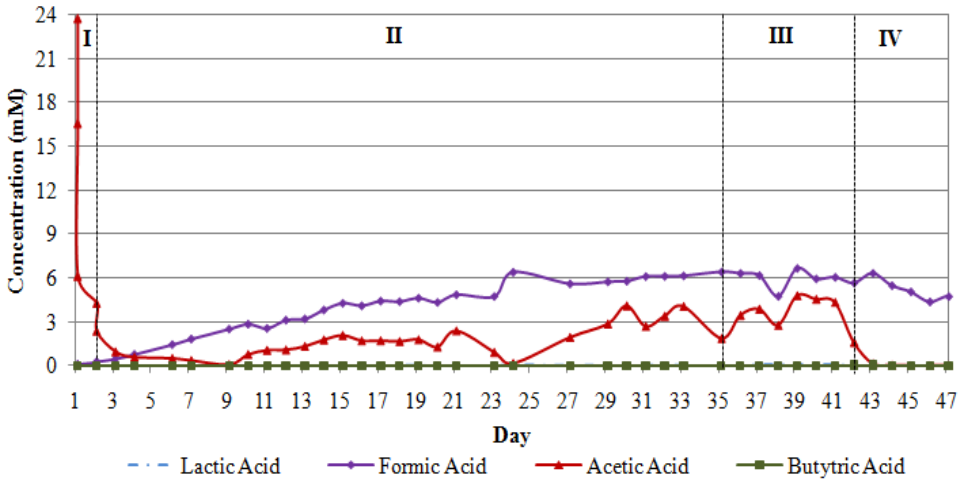


Figure 4.21 Organic acid utilization in the 40 mM/2 mM Ac/Glu fed CPBR experiment (18th September, 2008 - 2nd November, 2008).

4.3.2 Feed Composition: 40mM Acetate and 2 mM Glutamate with Biomass Recycle

The photobioreactor was started in indoor conditions using the procedure stated in Section 3.4.3. 25% bacterial inoculation and hydrogen production medium containing acetate (40 mM) and glutamate (2 mM) were used at startup. Continuous feeding was done with the same hydrogen production feed medium.

During continuous operation of the photobioreactors, as described in Section 3.4.4, 800 ml bacterial effluent was initally removed and 800 ml hydrogen production medium was fed into system. The removed bacteria effluent was chemically sterilized and discarded. However, in the biomass recycling case, the bacteria effluent was centrifuged and the supernatant discarded while the pellet was dissolved in hydrogen production medium and fed back into the photobioreactor. The details are described in Section 3.6.1. Using the procedure described in Section 3.6.2, it was found that 0.23 g of biomass was recycled back into the photobioreactor.

Shown in Figure 4.22 is the biomass growth and hydrogen production for the 40 mM/2 mM Ac/Glu CPBR with biomass recycle. In Phase I, after inoculation, due to constant illumination (2500 lux~143W/m^2) and controlled temperature, the bacteria grew steadily (μ =0.021 h^{-1}) and reached stationary Phase on the 5th day. 0.749 gdcw/l_c biomass concentration and 14 ml H_2/l_c.h hydrogen productivity was attained in this phase. The continuous feed with biomass commenced on the 5th day. As seen in Phase II, biomass concentration ranged between 0.55-0.65 gdcw/l_c. Stable hydrogen production at a rate of 11 ml H_2/l_c.h was obtained in this phase.

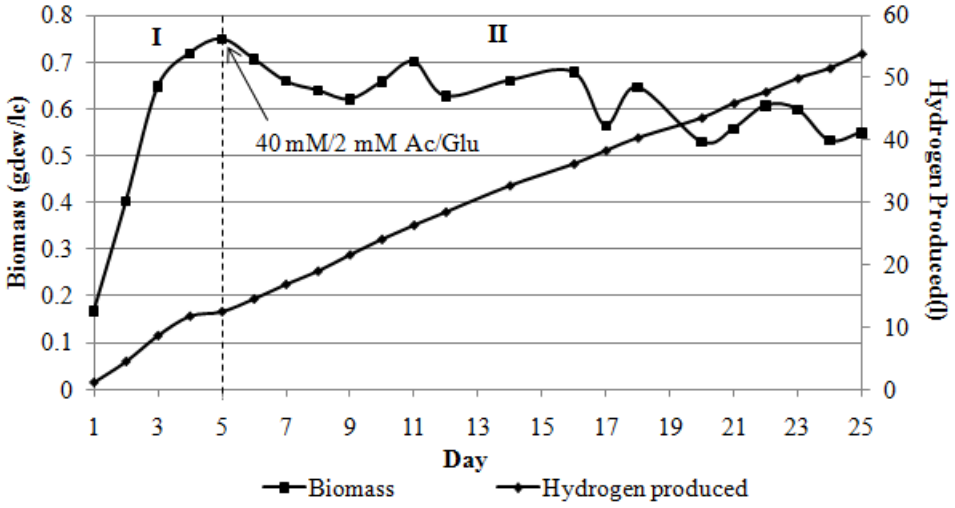


Figure 4.22 The biomass growth and hydrogen production in the 40 mM/2 mM Ac/Glu fed CPBR with biomass recycle (4th April, 2009 – 5th May, 2009).

During the experiment, an average pH of 7 and bacteriochlorophyll of 10 mg_{bchl}/l_c was obtained. Bacteriochlorophyll is the bacteria's photosynthetic pigment that is used to trap light at different wavelengths. It remained stable because of the constant illumination provided by the 60W tungsten lamps. The photobioreactor temperature was maintained between 30-35 ^{0}C as shown in Figure 4.23.

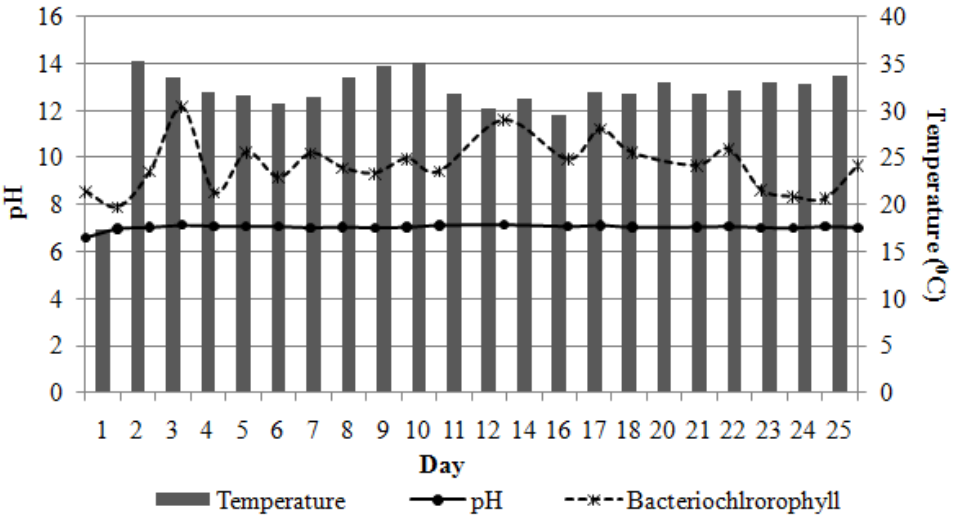


Figure 4.23 Temperature, pH and bacteriochlorophyll variation in the 40 mM /2 mM Ac/Glu fed CPBR with biomass recycle (4th April, 2009 – 5th May, 2009).

In the organic acid utilization in Figure 4.24, it is seen that acetic acid was steadily consumed at the startup (Phase I). 95% acetic acid conversion, 45% substrate conversion efficiency and 0.45% light conversion was obtained in this phase. In Phase II, with continuous feeding and biomass recyle, the acetic acid concentration averaged 2 mM. For the 20 days period in Phase II, 95% acetic acid conversion, 67% substrate conversion efficiency and 0.45% light conversion was obtained. Shown in Table 4.3 is a summary of the 40 mM/2 mM Ac/Glu CPBR with biomass recycle experiment results.

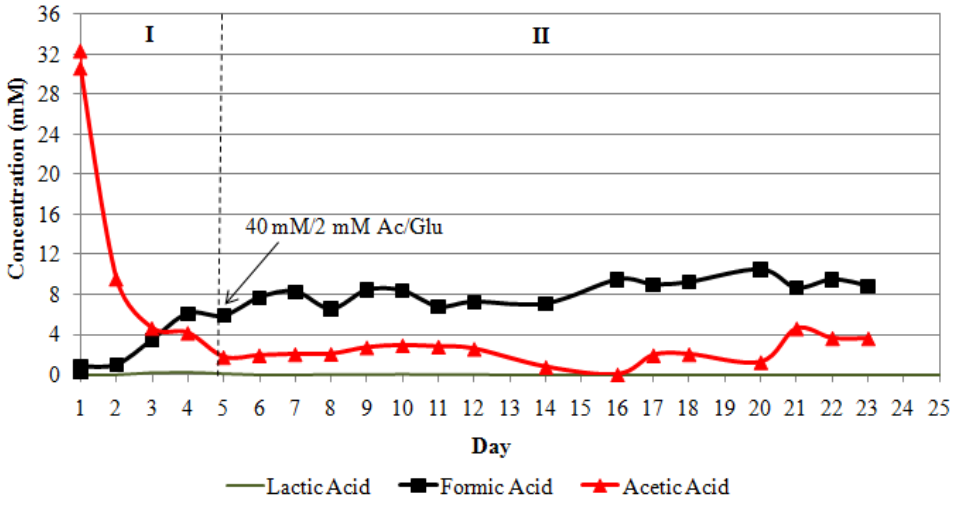


Figure 4.24 Organic acid utilization in the 40 mM/2 mM Ac/Glu fed CPBR with biomass recycle (4th April, 2009 – 5th May, 2009).

4.3.3 Feed Composition: 40 mM Acetate and 3-4 mM Glutamate

This experiment aimed to obtain a stable biomass and hydrogen production by changing the glutamate concentration while keeping the acetic acid concentration in the hydrogen production medium constant at 40 mM. An 8 l indoor photobioreactor was started as described in section 3.4.3. 22% bacterial inoculation and hydrogen production medium containing acetate (40 mM) and glutamate (2 mM) was used at the startup. Continuous illumination (2500 lux~143W/m^2) was provided by four 60 W tungsten lamps and controlled temperature using an air conditioner. The biomass concentration and hydrogen production are shown in Figure 4.25.

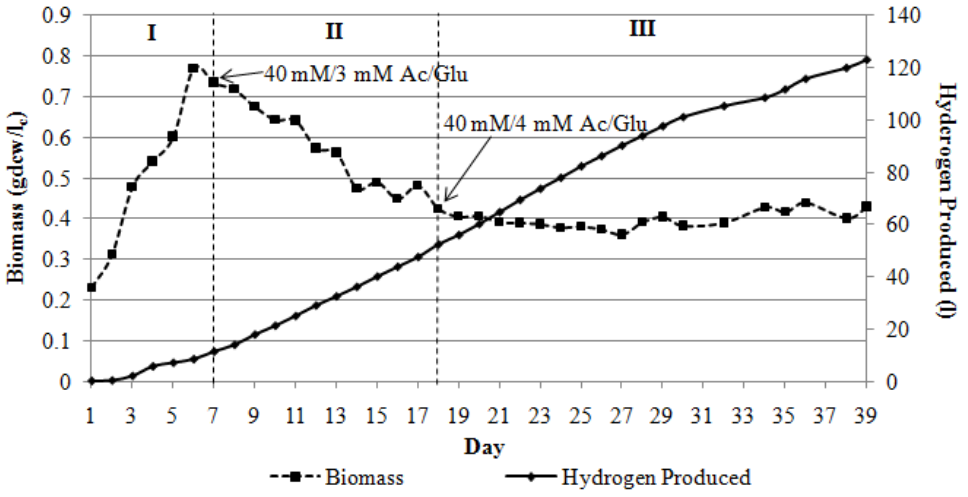


Figure 4.25 The biomass growth and hydrogen production in the 40 mM/3 mM – 40 mM/4 mM Ac/Glu fed CPBR experiment (25th March – 3rd May, 2009).

In Phase I, the bacteria grew steadily (μ= 0.019 h^{-1}) and reached a biomass concentration of 0.771 gdcw/l$_c$ in 6 days as seen in Figure 4.23. Hydrogen production medium containing 40 mM acetate and 2 mM glutamate was used in this phase. 14 ml H_2/l$_c$.h hydrogen production with 62% acetate conversion and 58% substrate conversion efficiency was obtained.

In Phase II, continuous feeding using 40 mM acetate and 3 mM glutamate was introduced. Despite increasing the glutamate amount in the feed, biomass decreased to 0.45 gdcw/l$_c$. Higher hydrogen production rate 18 ml H_2/l$_c$.h with 84 % acetate conversion, 105 % substrate conversion efficiency and 0.66 % light conversion efficiency was obtained. The high acetate conversion and substrate conversion efficiency showed that the bacteria used most of the acetate for hydrogen production. Moreover, the over 100% exceed of the substrate conversion is because the bacteria did not only utilize acetate and glutamate but also other sources like the side products (PHB) and biomass in the culture for hydrogen production. Sasikala *et al*. (1990), observed over 100% substrate conversion efficiency using *R. sphaeroides* and malate as feed.

They attributed this to the existence of endogenous substrates which acted as electron donors for hydrogen production. Endogenoous substrates are known to act as electron donors for hydrogen formation (Ormerod *et al*., 1961; Hillmer and Gest, 1977). To counteract further biomass decrease, the glutamate in the daily 800 ml hydrogen production feed was increased to 4 mM.

In Phase III, it was observed that biomass stabilized at 0.4 gdcw/l_c. 17 ml H_2/l_c.h hydrogen production rate with 98% acetate conversion, 85% substrate conversion efficiency and 0.70 % light conversion efficiency was obtained. Gas analysis showed that 87-92% hydrogen was produced in the three phases. An average pH of 7 and 9 mg_{bchl}/l_c bacteriochlorophyll was also obtained. The variation in temperature, pH and bacteriochlorophyll are shown in Figure 4.26.

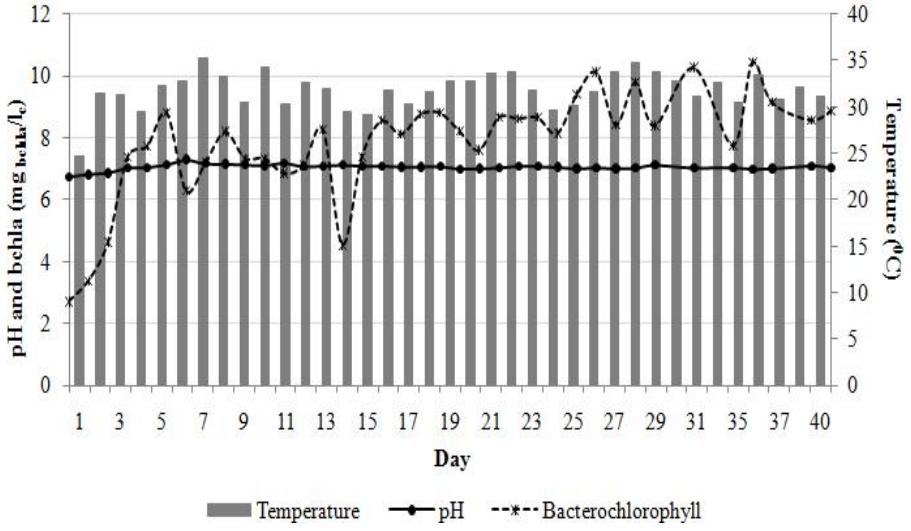


Figure 4.26 Temperature, pH and bacteriochlorophyll variation in the 40 mM/3 mM- 40 mM/4 mM Ac/Glu fed CPBR experiment (25th March – 3rd May, 2009).

The organic acid utilization plot Figure 4.27 shows that acetic acid was almost completely used in Phase III where stable biomass was attained. Lactic, butyric and propionic acids were formed in low quantities but, formic acid was formed and accumulated in the system. It is observed that the increase in glutamate amount led to an increase in formic acid formation. An average of 10 mM formic acid was obtained in Phase III. Shown in Table 4.3 is a summary of the 40 mM/3 mM - 40 mM/4 mM Ac/Glu continuous panel photobioreactor.

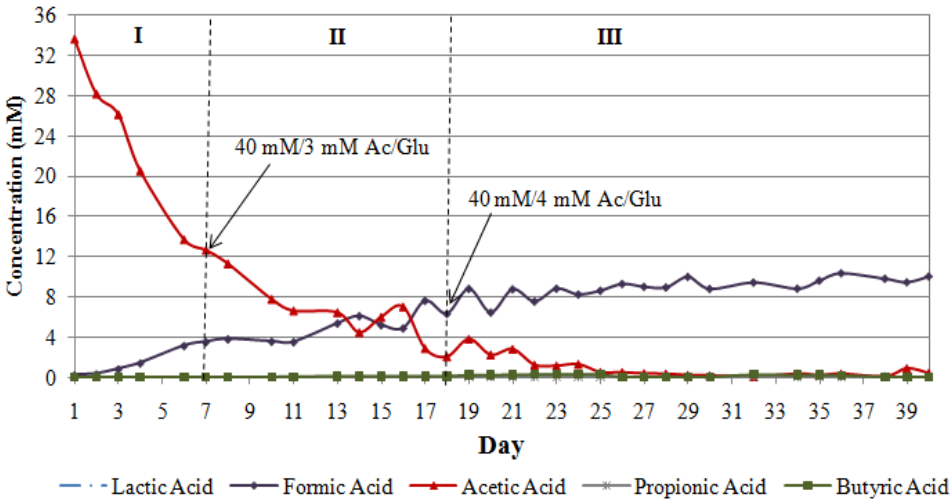


Figure 4.27 Organic acid utilization in the 40/3-40/4 mM Ac/Glu fed CPBR experiment (25th March – 3rd May, 2009).

4.3.4 Feed Composition: 60 mM Acetate and 3- 4 mM Glutamate

The indoor continuous panel photobioreactor was started using the procedure described in section 3.4.3. At the startup, 24% bacterial inoculation and 40 mM/2 mM Ac/ Glu containing hydrogen production medium was used. The 8 l panel photobioreactor was constantly illuminated (2500lux~143W/m^2)

using four 60W tungsten lamps and the room temperature controlled using an air conditioner. 10% dilution rate was used in the continuous operation.

The CPBR biomass growth and the hydrogen production is shown in Figure 4.28. In Phase I, the bacteria was grown using 40 mM/2 mM Ac/Glu containing hydrogen production medium. It grew steadily (μ= 0.027 h^{-1}) and reached 0.68 gdcw/l_c on the 4th day. 7 ml H_2/l_c.h of hydrogen gas was also produced. In Phase II, continuous feeding began and the feed composition was changed to 60 mM/3 mM Ac/ Glu. Stable biomass concentration at 0.68 gdcw/l_c was observed for 8 days after feeding commenced but a decrease to 0.53 gdcw/l_c was obtained on the 22nd day of the experiment. Stable hydrogen production at 11 ml H_2/l_c.h was got. In Phase III, to prevent further decrease in the biomass, the daily fed hydrogen composition was changed to 60/4 mM Ac/Glu. Biomass stabilized at 0.55 gdcw/l_c and 12 ml H_2/l_c.h hydrogen production rate was attained.

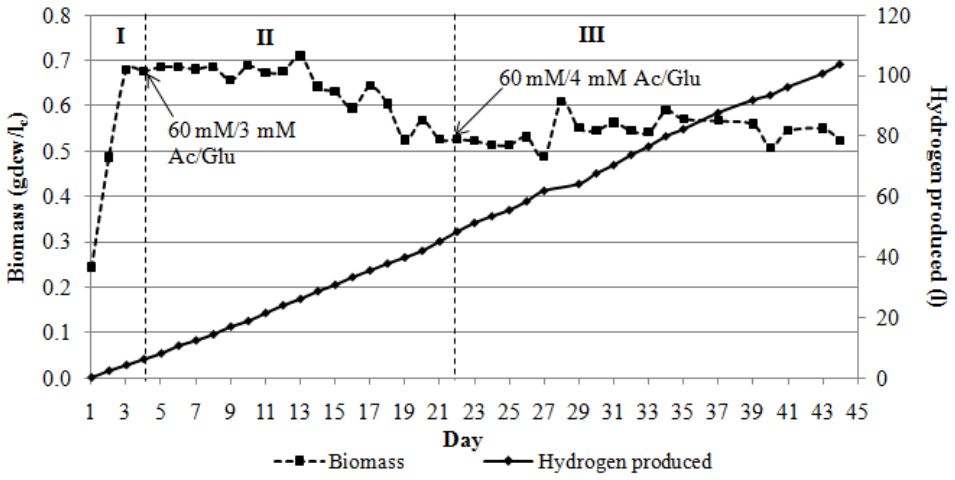


Figure 4.28 The biomass growth and hydrogen production in the 60 mM/3 mM – 60 mM/4 mM Ac/Glu fed CPBR experiment (21st March – 3rd May, 2009).

The variation in the pH, bacterichlorophyll and temperature is shown in Figure 4.29. An average pH of 7, 8 mg bchl/l_c bacterichlorophyll and 31^{0}C average temperature was obtained.

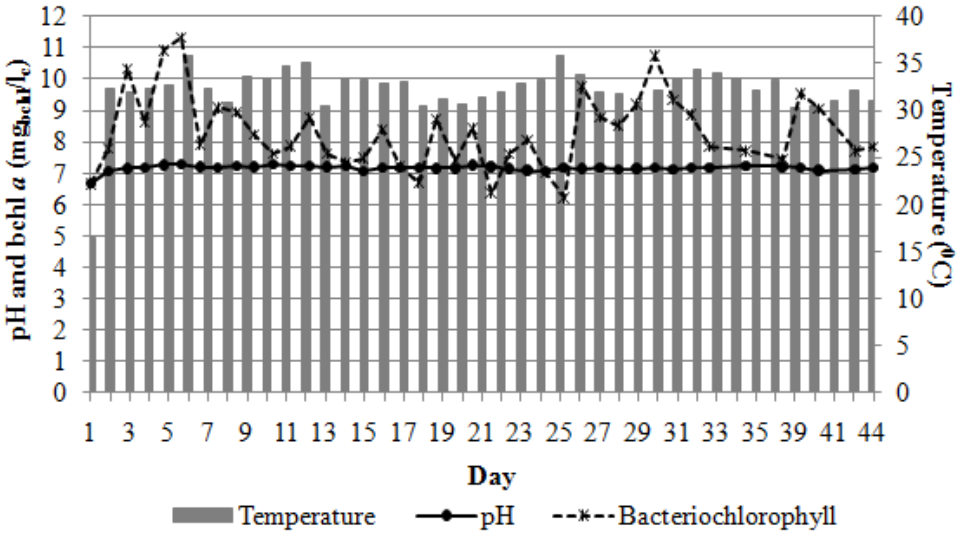


Figure 4.29 Temperature, pH and bacteriochlorophyll variation in the 60 mM/3 mM – 60 mM/4 mM Ac/Glu fed CPBR experiment (21st March – 3rd May, 2009).

From the organic acid utilization in Figure 4.30, accumulation of acetic acid in the system is observed. In Phase II, with the 60 mM/3 mM Ac/Glu feed, 22 mM of acetic acid accumulated in the system. 67 % acetate conversion and 57% substrate conversion efficiency was obtained. The bacteria utilized the acetic acid given for hydrogen production but the glutamate provided was not enough to maintain a stable biomass. In Phase III, with the increase in the glutamate amount in the feed from 3 mM to 4 mM, biomass stabiled at 0.55 gdcw/l_c. 70 % acetate conversion and 61% substrate conversion efficiency were obtained. The accumulated acetic acid in the system decreased to 14 mM; it was used for growth and hydrogen production. Formic acid formation

increased with the increase in glutamate amount. Lactic, propionic and butyric acid were formed in low concentrations. A summary of the 60 mM/3 mM - 60 mM/4 mM Ac/Glu experiment is given in Table 4.3.

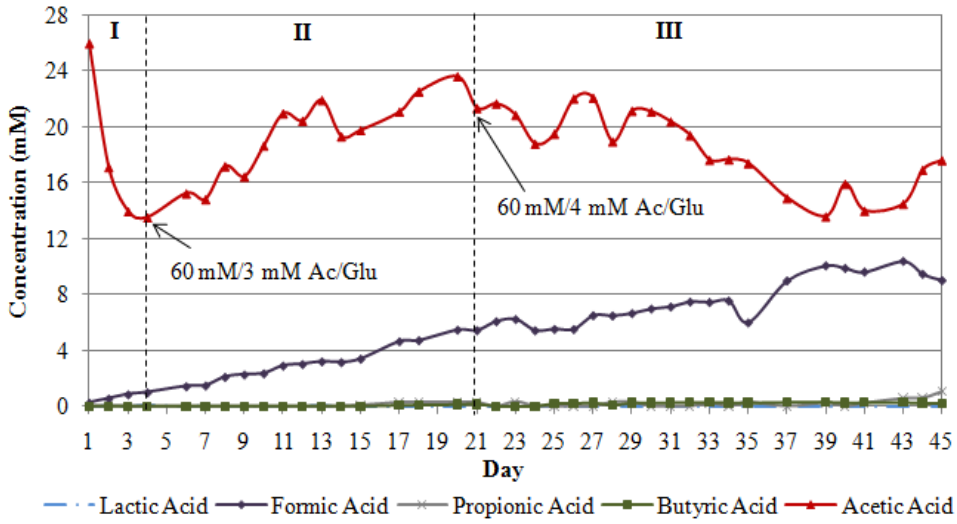


Figure 4.30 Organic acid utilization in the 60 mM/3 mM – 60 mM/4 mM Ac/Glu fed CPBR experiment (21st March – 3rd May, 2009).

4.3.5 Feed Composition: 80 mM Acetate and 4 mM Glutamate

An experiment to investigate the effect of increasing the acetate and glutamate amount keeping the C/N ratio constant at 45 (as in the 40 mM/2 mM and 60 mM/3 mM CPBR experiment) was carried out. The photobioreactor was started using the procedure described in section 3.4.3 with 23 % bacterial inoculation and 40 mM/2 mM Ac/Glu hydrogen production medium. Daily 800 ml continuous feeding of the 80 mM/4 mM Ac/Glu hydrogen production medium started on the 3rd day when the biomass concentration was 0.9 gdcw/l_c. Stable biomass at 1 gdcw/l_c and 12 ml H_2/l_c.h hydrogen production was

attained in Phase II. The biomass growth and cumulative hydrogen production is shown in Figure 4.31.

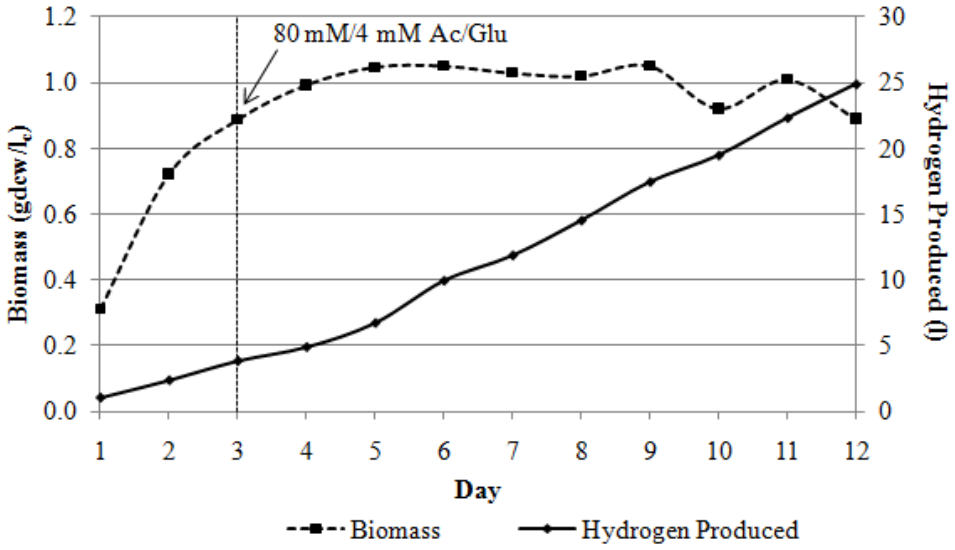


Figure 4.31 The biomass growth and hydrogen production in the 80 mM/4 mM Ac/Glu fed CPBR experiment (21st March, 2009 – 1st April, 2009).

An average pH 7 and 12 mg bchl/l$_c$ bacterichlorophyll was obtained. The photobioreactor temperature was maintained at 31^{0}C on average as shown in Figure 4.32.

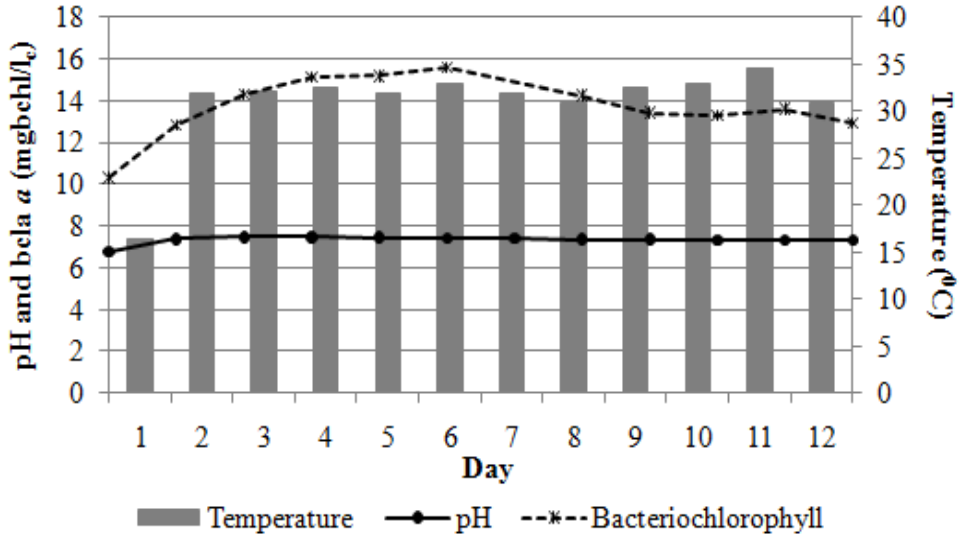


Figure 4.32 Temperature, pH and bacteriochlorophyll variation in the 80 mM/4 mM Ac/Glu fed CPBR (21st March, 2009 – 1st April, 2009).

Organic acid analyses using HPLC (Figure 4.33) showed that acetic acid was utilized for growth in Phase I. 54% acetate conversion and 17 % substrate conversion efficiency was obtained in this phase. Accumulation of acetic acid was observed on the onset of continuous feeding in Phase II. Acetate conversion and substrate conversion efficiency were 75% and 39% respectively. The accumulation was due to the excess acetic acid given during feeding. Glutamic acid measurement showed that the bacteria fully utilized glutamate for growth at startup and to maintain biomass at the onset of continuous feeding. This nitrogen limited condition is desired for hydrogen production.

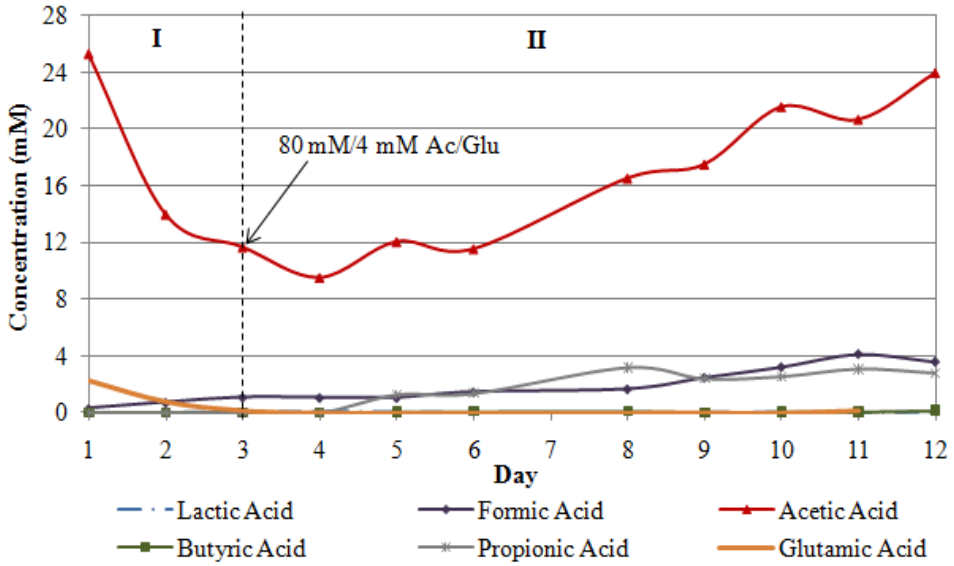


Figure 4.33 Organic acid utilization in the 80 mM/4mM Ac/Glu fed continuous panel photobioreactor (21st March, 2009 – 1st April, 2009).

4.4 Long Term Stability of Biomass

4.4.1 Effect of Biomass Recycle

The 40 mM/2 mM Ac/Glu fed photobioreactors with and without biomass recycle are compared. Similar biomass change was observed in the two photobioreactors as shown in Figure 4.34. Despite having initial different growth rates, 0.021 $h^{-1}$and 0.061 h^{-1} for the CPBR with and without biomass recycle respectively, biomass concentration decreased to 0.5 gdcw/l_c at the end of the 25th day. This showed that the biomass recycle was insufficient in curbing the decrease in biomass problem. It asserts the need to adjust the feed composition for stable biomass and hydrogen production.

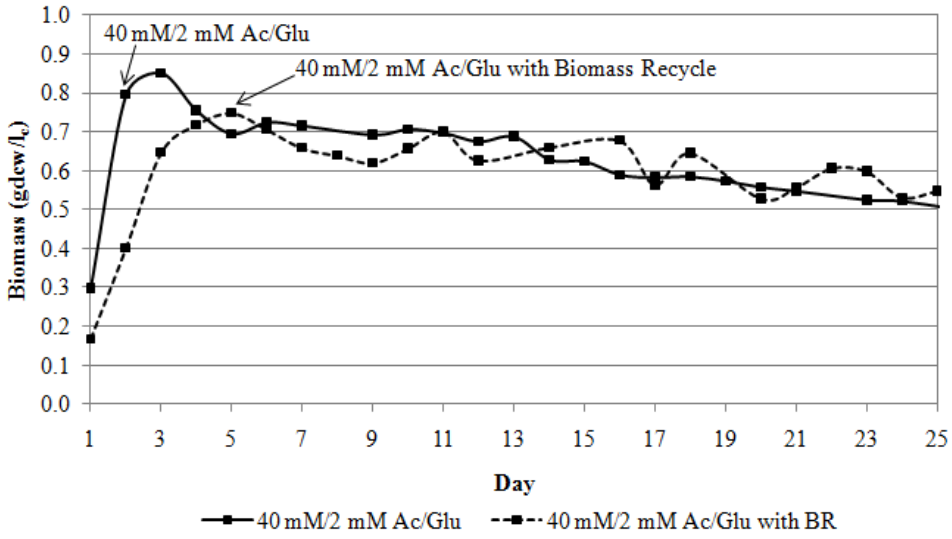


Figure 4.34 Effect of biomass recycle on biomass concentration.

In Figure 4.35 (a), it is seen that the 40 mM/2 mM Ac/Glu fed CPBR had a higher molar hydrogen productivity (0.7 mmol $H_2/l_c.h$) compared to the 40 mM/2 mM Ac/Glu fed CPBR with biomass recycle (0.5 mmol $H_2/l_c.h$). High acetate conversion was observed in both CPBRs; 96% in the 40 mM/2 mM Ac/Glu fed CPBR and 95% in the 40 mM/2 mM Ac/Glu fed CPBR with biomass recycle. This showed that acetate was well utilized in both photobioreactors. However, lower substrate conversion efficiency was obtained in the biomass recycle case as seen in Figure 4.35 (c). A reason for this was that part of the acetate fed was used by the recycled biomass for maintenance purposes.

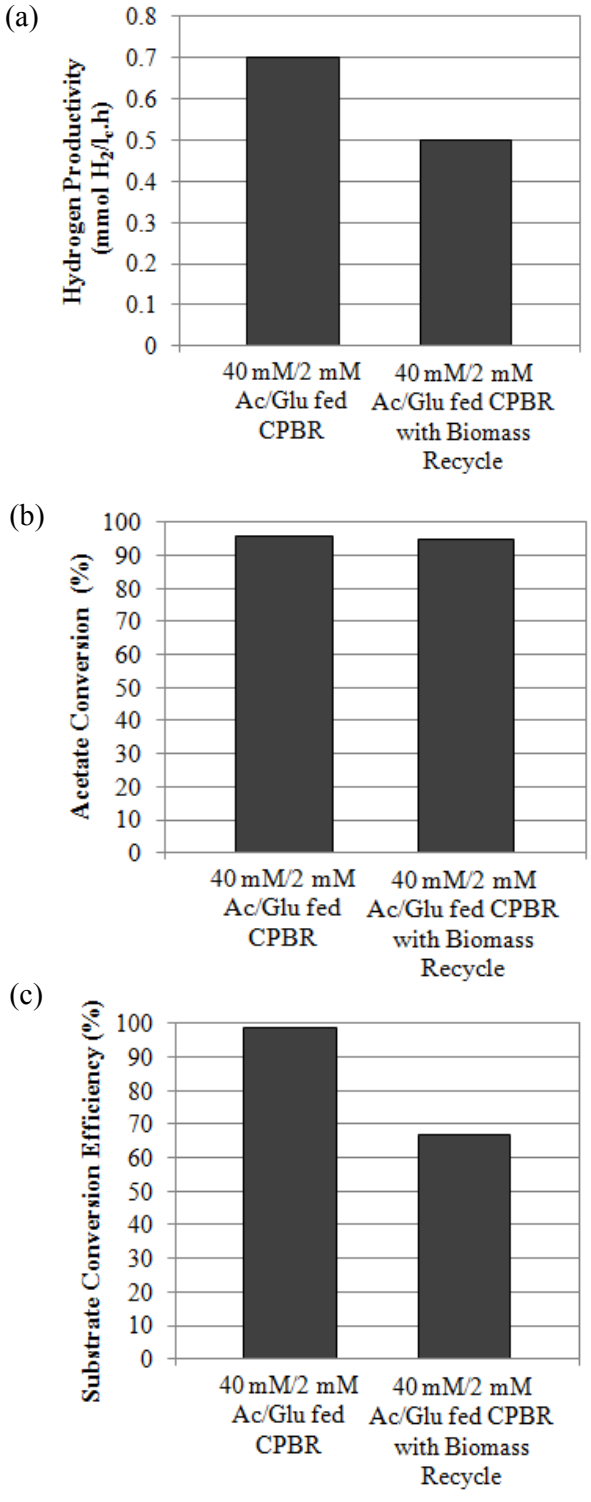


Figure 4.35 Effect of biomass recycle on: (a) Hydrogen productivity, (b) Acetate conversion and (c) Substrate conversion efficiency.

4.4.2 Effect of Increasing Glutamate Concentration in the Feed keeping Acetate Concentration Constant

Glutamate contains nitrogen that is used by the bacteria for growth purposes. Therefore, the major effect of the glutamate increase was on biomass formation. The 40 mM/2 mM Ac/Glu and 40 mM/3 mM – 40 mM/4 mM Ac/Glu fed continuous photobioreactors are compared in Figure 4.36. It was observed that the increase in glutamate amount in the feed led to a stable biomass growth during the continuous operation of the photobioreactor. Decrease of biomass was observed in the 40 mM /2mM and 40 mM/3 mM Ac/Glu feed composition; however stable biomass at 0.40 gdcw/l_c was achieved using the 40 mM/4 mM Ac/Glu feed. These results validate the suitability of using glutamate as a nitrogen source as reported by Eroğlu *et al*. (1999) and Koku *et al*. (2002).

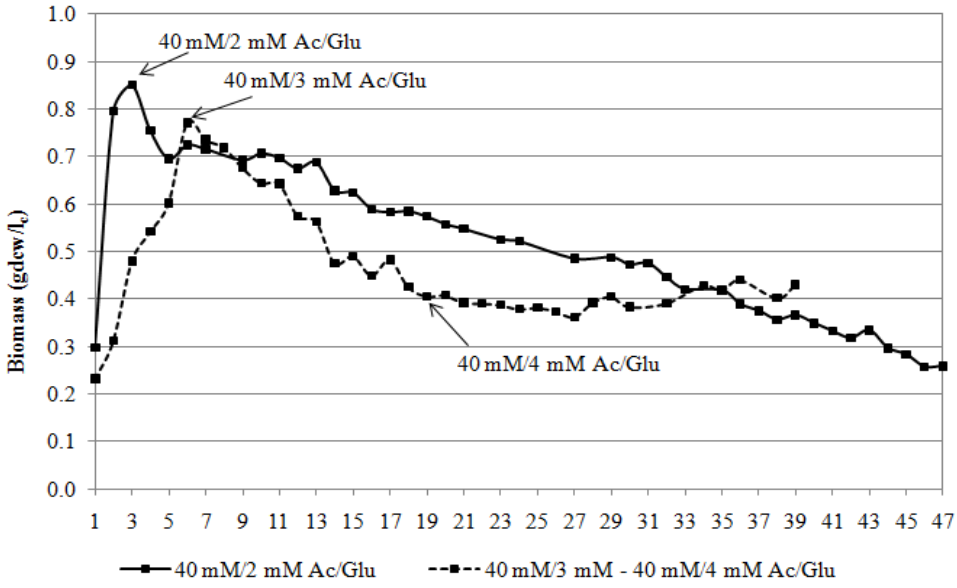


Figure 4.36 Biomass change with increasing glutamate concentration keeping acetate concentration constant.

4.4.3 Effect of Increasing Acetate Concentration in the Feed keeping Glutamate Concentration Constant

Higher biomass concentrations were observed with increasing acetate concentrations. 0.40 gdcw/l_c, 0.54 gdcw/l_c and 1 gdcw/l_c biomass concentrations were obtained in the 40 mM/4 mM, 60 mM/4 mM and 80 mM/4 mM Ac/Glu fed phases as shown in the Figure 4.37. Uyar (2007) observed that an increase in acetate did not affect hydrogen production but led to significant cell growth using *R. sphaeroides*. Tao *et al*. (2007) reported that an acetate threshold level at 15 mM existed for *R. sphaeroides* SH2C. They did not observe hydrogen production when acetate concentration was below 15 mM, but reported hydrogen production when the acetate concentration was increased to 50 mM.

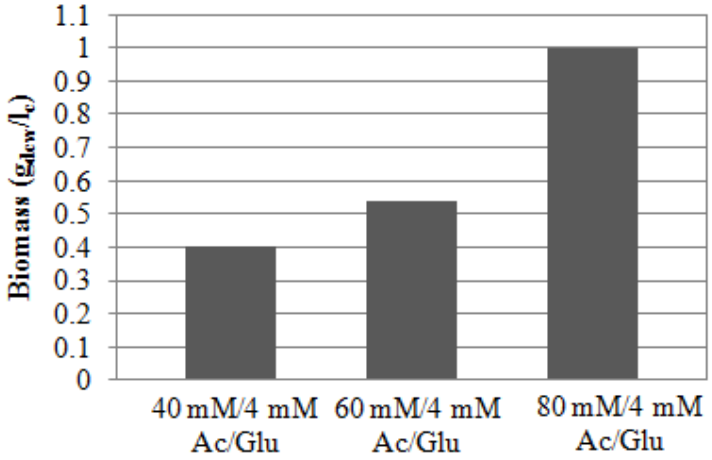


Figure 4.37 Biomass change with increasing acetate concentration keeping glutamate concentration constant.

4.5 Evaluation of the Results

4.5.1 Effect of the C/N Ratio

The C/N ratio in the feed media was reported as a critical parameter for hydrogen production process (Eroğlu *et al*., 1999). The effect of increasing glutamate and acetate amount in the feed (changing the C/N ratio) and maintaining the C/N constant while increasing the feed concentrations was investigated. The results were evaluated with regards to hydrogen productivity, acetate conversion and substrate conversion efficiency. Shown in Figure 4.38 are the hydrogen productivities, substrate conversion efficiencies and the acetate conversions for the indoor CPBRs.

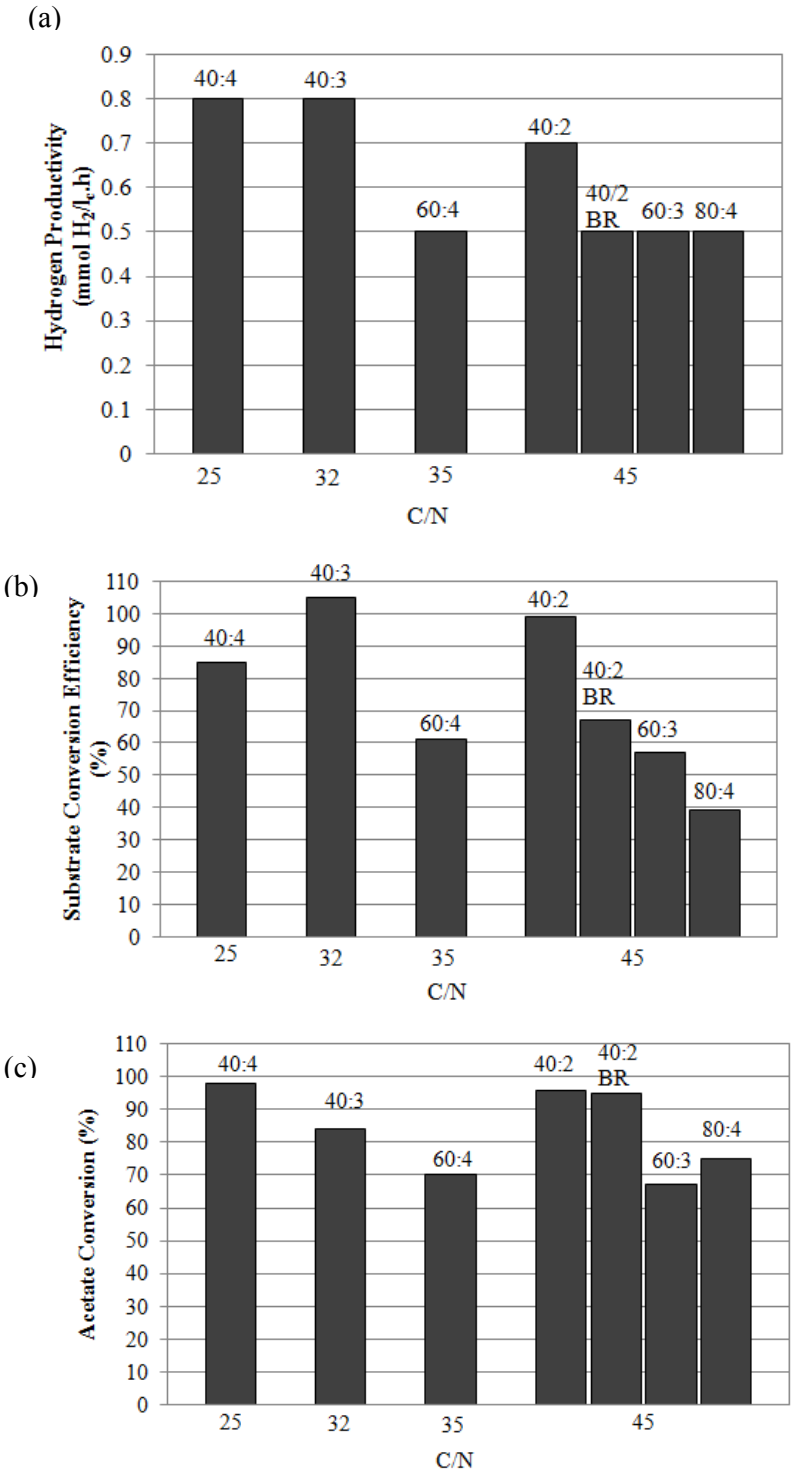


Figure 4.38 (a) Hydrogen productivity, (b) Substrate conversion efficiency and (c) Acetate conversion in the indoor photobioreactors. (40:4 stands for 40 mM Ac/ 4 mM Glu).

The effect of increasing the glutamate amount in the feed media (decreasing the C/N ratio) is discussed considering the feed media compositions; 40 mM/2 mM Ac/Glu (C/N=45), 40 mM/3 mM Ac/Glu (C/N=32) and 40 mM/ 4 mM (C/N=25)

An increase in hydrogen productivity was observed with increasing glutamate concentration. Higher hydrogen productivities of 0.7 mmol $H_2/l_c.h$, 0.8 mmol $H_2/l_c.h$ and 0.8 mmol $H_2/l_c.h$ were obtained in the 40 mM/2 mM Ac/Glu, 40 mM/3 mM Ac/Glu and 40 mM/ 4 mM photobioreactors respectively as seen in Figure 4.38 (a). Also, higher substrate conversion efficiencies were obtained with increasing the feed media's glutamate amount, keeping acetate constant. As seen in Figure 4.38 (b) high substrate conversion efficiencies of 99%, 105% and 84% were attained for the 40 mM/2 mM Ac/Glu, 40 mM/3 mM Ac/Glu and 40 mM/ 4 mM fed CPBR respectively. 105% substrate conversion efficiency observed in the 40 mM/3 mM Ac/Glu could be attributed to the existence of endogenous sources which the bacteria could use for hydrogen production. The bacteria did not only use acetate and glutamate for growth (maintenance) and hydrogen production, but also other endogenous sources such as PHB as well. Sasikala *et al*. (1990) observed over 100% substrate conversion efficiency using *R. sphaeroides* and malate as feed. They attributed this to the existence of endogenous substrates which acted as electron donors for hydrogen production. Endogenous substrates are known to act as electron donors for hydrogen formation (Ormerod *et al*., 1961; Hillmer and Gest, 1977). This theory is further validated by the lower acetate conversion (84%) observed in the 40 mM/3 mM case, compared to the 40 mM/2 mM and 40 mM/4 mM Ac/Glu cases as seen in Figure 4.38 (c). Due to the utilization of these other sources for hydrogen production, less acetate was consumed and so decreasing the acetate conversion values. The highest acetate conversion (98%) was observed in the 40 mM/4 mM Ac/Glu with stable biomass.

Besides the increase in hydrogen productivity, substrate conversion efficiency and acetate conversion, another effect of glutamate increase (C/N

ratio decrease) observed was on the formation of formic acid. Looking at the HPLC results in Figures 4.21 and 4.27, it is observed that formic acid averaged at 6 mM in the 40 mM/2 mM Ac/Glu CPBR but increased to 10 mM in the 40 mM/4 mM case. Eroğlu *et al*. (2008) observed that in outdoor conditions, formate was formed during the night and utilized in daytime. It can be inferred that the formate concentration reached a steady state concentration because of the constant low light illumination and dilution rate. Increasing the light intensity would alleviate the formate concentration. Uyar *et al*. (2007) reported increasing hydrogen production with increasing light intensity and obtained hydrogen production saturation at 270 W/m^2. In the indoor experiments carried out in this study, the light intensity was 143 W/m^2.

Moreover, gas analysis showed a slight increase in the carbondioxide levels with the increase in glutamate concentration. Hydrogen levels in the gas composition decreased to 87% with 13% being carbondioxide.

The C/N ratio was also changed by increasing the acetate concentration while maintaining the glutamate amount constant. Özgür *et al*. (2009) tested the effect of increasing initial acetate concentration (10-50 mM) on hydrogen production and biomass using *R. capsulatus* YO3 (Hup^-) in batch PBR. They observed growth and hydrogen production in all samples but obtained the highest hydrogen productivity with the 40 mM/2 mM Ac/Glu. Similarly, in this study, the highest hydrogen productivity was obtained with the 40 mM/4 mM Ac/Glu feed, as shown in Figure 4.37 (a). A further increase in acetate, as in the 60 mM/4 mM Ac/Glu and 80 mM/4 mM Ac/Glu feed media, led to a decrease in hydrogen productivity.

An increase in acetate concentration also led to a decrease in substrate conversion efficiency. This is anticipated as the HPLC analyses in Figures 4.27, 4.30 and 4.33 show that while acetate was almost completely utilized in the 40 mM/4 mM Ac/Glu, accumulation was observed in the 60 mM/4 mM and 80 mM/4 mM Ac/Glu cases. Highest substrate conversion efficiency and acetate conversion was observed in the 40 mM/4 mM Ac/Glu. In Figure 4.38 (b) substrate conversion efficiency decreased with increasing

acetate concentration while acetate conversion remained more or less the same. This depicts that there exists an acetate concentration limit during operation of continuous systems at stable biomass concentration. Above this maxima limit, acetate accumulation occurs. For photobioreactors using *R. caspulatus* YO3 (Hup^-) and hydrogen production medium containing 4 mM glutamate, this limit is found to be at 40 mM acetate.

Another effect of C/N ratio investigated is the keeping of the C/N ratio constant while increasing the acetate and glutamate concentrations. The photobioreactors fed by 40 mM /2 mM Ac/Glu, 60 mM /3 mM Ac/Glu and 80 mM /4 mM Ac/Glu (all having C/N= 45) are compared.

Higher biomass concentration was obtained with increasing feed component concentrations keeping C/N constant as shown in Figure 4.39

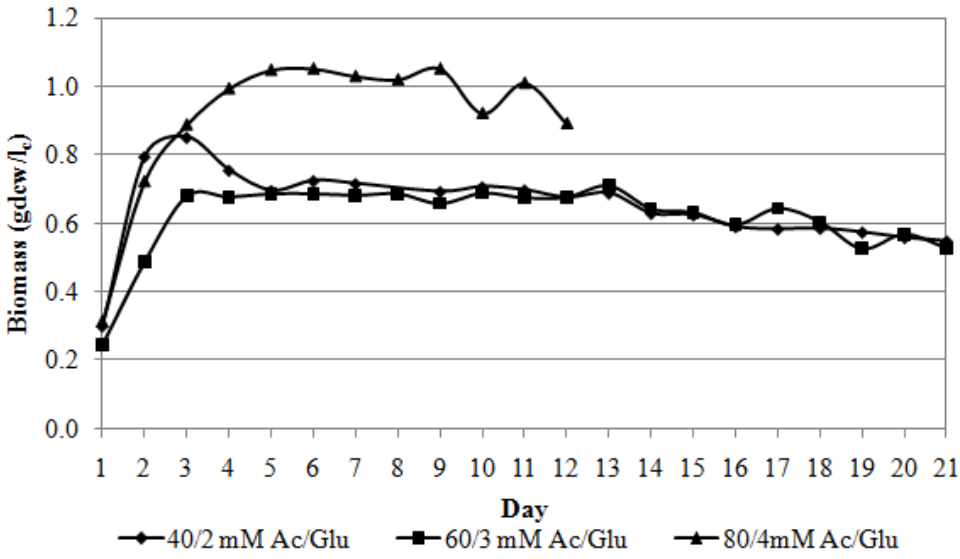


Figure 4.39 Effect of increasing acetate and glutamate keeping C/N constant on biomass.

The substrate conversion efficiency was found to decrease with increasing feed concentrations. HPLC analysis results in Figures 4.21 show that acetate was well utilized in the 40 mM/2 mM Ac/Glu CPBR. In Figures 4.30 and 4.33, accumulation of acetic acid in the 60 mM /3 mM Ac/Glu and 80 mM /4 mM Ac/Glu CPBRs was observed which led to lower acetate conversions as seen in Figure 4.38 (c). The highest acetate conversion (96%) was obtained in the 40 mM /2 mM Ac/Glu. Also, the highest hydrogen productivity was got in the 40 mM /2 mM Ac/Glu photobioreactor as shown in Figure 4.38 (a).

Overall, 25 C/N feed composition (40 mM/4 mM Ac/Glu) ratio was found to be optimum for stable biomass growth and hydrogen production in the *R. caspulatus* YO3 (Hup^-) containing CPBRs.

4.5.2 Light Conversion Efficiencies in the Indoor Continuous Photobioreactors

The photosynthetic efficiency of photoheterotrophic hydrogen formation when sunlight or tungsten illumination is used is in the range 0.5-6% (Otsuki *et al*., 1998; Steinborn and Oelze, 1989; Sasaki *et al*., 1998; Zürrer and Bachofen, 1982; Hoekama, 2000). The light conversion efficiencies of the indoor experiments in this study range between 0.24-1.21% as shown in Figure 4.40. Lower light efficiencies were observed with increasing glutamate and acetate concentrations. An explanation for this could be the increase in biomass and the decrease in the amount of hydrogen produced.

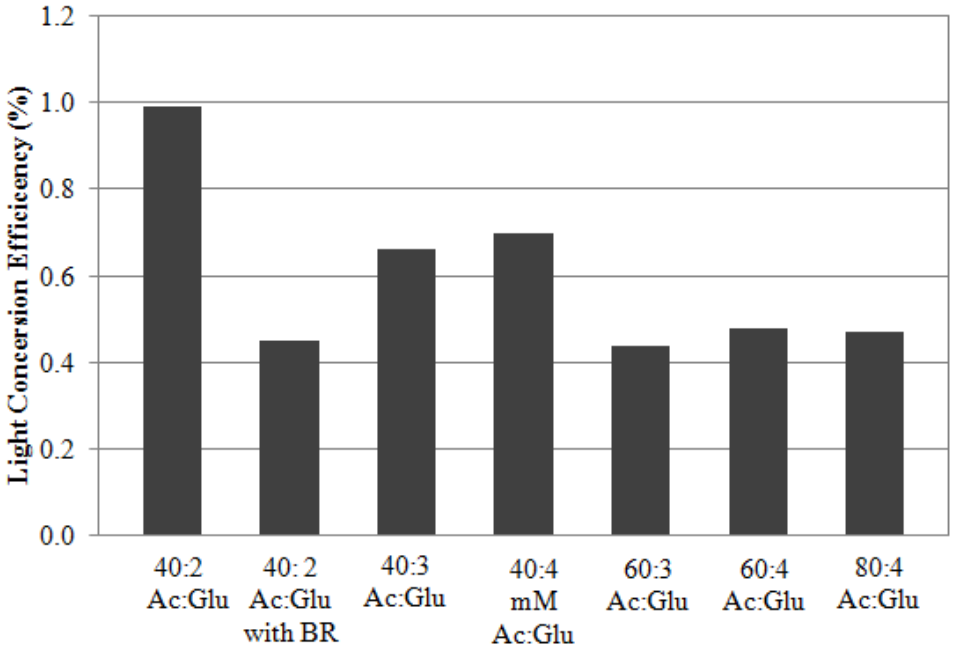


Figure 4.40 Light conversion efficiency values for the *R. capsulatus* YO3 (Hup$^-$) indoor CPBRs.

4.5.3 Comparison of Batch and Continuous Photobioreactors

The indoor 40 mM/2 mM Ac/Glu CPBR carried out using *R. capsulatus* YO3 (Hup$^-$) is compared to an indoor batch experiment using 40 mM/2 mM Ac/Glu reported by Özgür *et al*. (2009).

In Figures 4.41 (a), (b) and (c), it is observed that the continuous systems have a higher hydrogen productivity, substrate conversion efficiency and light conversion efficiency compared to the batch systems. This difference could be because of the replenishment of used carbon and nitrogen sources through feeding in the continuous system. This ascertains the use of continuously operated photobioractors to batch systems for continuous hydrogen production.

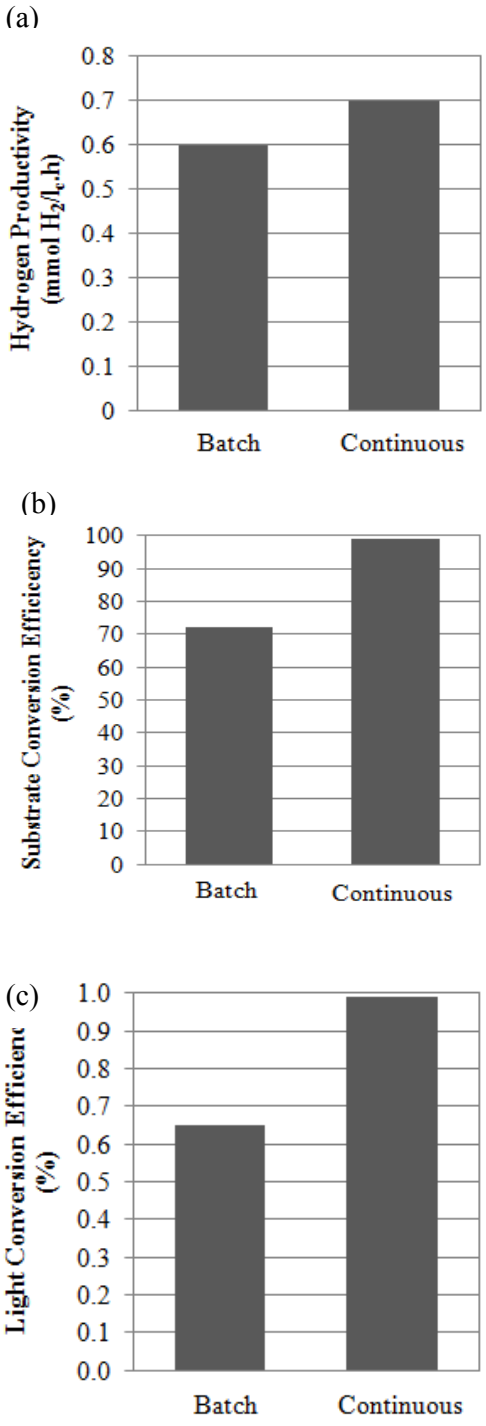


Figure 4.41 Comparison of batch versus continuous photobioreactors:
(a) Hydrogen productivitiy, (b) Substrate conversion efficiency
(c) Light conversion efficiency.

Table 4.3 Summary results of the indoor continuous panel photobioreactors with *R. capsulatus* YO3 (Hup$^-$).

CPBR	Phase	Days	Temperature (^{0}C)	Biomass (g_{dcw}/l_c)	Hydrogen Productivity		Substrate Conversion Efficiency (%)	Acetate Conversion (%)	Light Conversion Efficiency (%)
					ml H_2/l_c.h	mmol H_2/l_c.h			
40 mM /2 mM Ac/Glu	I[a]	2	31 ± 5	0.80	9	0.4	20	82	0.75
	II	31	31 ± 2	0.60 ± 0.11	15	0.7	99	96	0.99
	III	8	32 ± 1	0.37 ± 0.03	16	0.7	90	91	1.21
	IV	6	32 ± 1	0.29 ± 0.03	6	0.3	19	100	0.42
40 mM/2 mM Ac/Glu with Biomass Recycle	I[a]	5	31 ± 5	0.75	14	0.6	48	95	0.45
	II	20	32 ± 1	0.62 ± 0.06	11	0.5	67	95	0.45
40 mM/3 mM – 40 mM/4 mM Ac/Glu	I[a]	6	31 ± 3	0.74	7	0.3	58	62	0.32
	II	12	31 ± 2	0.58 ± 0.10	18	0.8	105	84	0.66
	III	22	32 ± 1	0.40 ± 0.03	17	0.8	85	98	0.70
60 mM/3 mM – 60 mM/4 mM Ac/Glu	I[a]	4	30 ± 5	0.68	7	0.3	46	48	0.29
	II	18	33 ± 2	0.63 ± 0.06	11	0.5	57	67	0.44
	III	22	32 ± 1	0.54 ± 0.03	12	0.5	61	70	0.48
80 mM/4 mM Ac/Glu	I[a]	3	30 ± 6	0.90	6	0.3	17	54	0.24
	II	9	32 ± 1	1.0 ± 0.06	12	0.5	39	75	0.47

a) Startup period.

*A conversion factor of 0.0445 mmol/ml was used in determining the molar hydrogen productivity.

4.6 Ammonium Ion Removal

High ammonia concentrations repress nitrogenase expression and activity of photosynthetic bacteria (Gogotov, 1986 and Zorin, 1986). So for continuous hydrogen production using PNS bacteria under anerobic and nitrogen limited conditions, the removal of ammonia ions from the feed media is a prerequisite. Clinoptilolite zeolite has a high affinity for ammonia ions and so suitable for ammonia ions reduction.

4.6.1 Gördes Clinoptilolite Batch Pre-treatment

In Section 3.7.1 Gördes clinoptilolite batch pre-treatment procedure was described. The chemical analysis results of the pre-treated clinoptilolite are tabulated in Table 4.4.

Table 4.4 Chemical analysis results of pre-treated clinoptilolite.

Clinoptilolite Sample	**Exchangeable Ions**			
	Ca^{+2} %	Mg^{+2} %	K^{+1} %	NaO %
Original	0.083	0.073	0.062	1.08
Pretreated	0.057	0.071	0.061	3.58

From Table 4.4, it is seen that the sodium ion concentration in the clinoptilolite was increased from 1.08% to 3.6%. These results are comparable to that obtained by Aşiroğlu (2006) who increased sodium ion content in the clinoptilolite from 0.6% to 3.6% via batch pretreatment procedure.

4.6.2 Dark Fermenter Effluent of Sugar-Beet Thick Juice Molasses Ammonium Ion Reduction

10 mM NH_4Cl solution and DFE of sugar-beet thick juice molasses were batch treated using pre-treated Gördes clinoptilolite as described in Section 3.7.2.

Table 4.5 DFE of molasses treatment using pretreated clinoptilolite sample.

Sample	**$g_{zeolite}$/50 ml sample**	**Time (h)**	**Concentration (mM)**		**% NH_4^+ Reduced**
			Initial	Final	
NH_4Cl solution	1	17	10	0.75	93
	0.5	17	10	2.6	74
Sugar- Beet Thick Juice Molasses (DFE)	0.6	48	12.4	4.7	62

As seen in Table 4.5, ammonium ion was effectively removed from the prepared NH_4Cl solution and 62% of the ammonium in the sugar-beet thick juice molasses was removed using clinoptilolite. Higher amounts of ammonium ion can be removed with increasing the zeolite amount as observed in the NH_4Cl solution.

4.6.3 Total Nitrogen Measurement

The total nitrogen measurements results in Table 4.6 show that the 10 mg/l and 25 mg/l standard NH_4Cl solutions were measured with 17% and 9.2% errors respectively. These discrepancies could be attributed to measurement errors (weighing and dilution). It is also observed that clinoptilolite was able to remove 52% of the total nitrogen content in the sugar-beet juice molasses DFE sample as seen in Table 4.7.

Table 4.6 Results of the total nitrogen measurement of NH_4Cl solution.

NH_4Cl solution	**Total N (mM)**	**Error (%)**
10 mg/l (0.7 mM)	0.6	17
25 mg/l (1.8 mM)	1.62	9.2

Table 4.7 Results of the total nitrogen measurement of NH_4Cl solution and Sugar-beet juice molasses DFE.

DFE Sample	**Total N (mM)**	**Total % N Reduced**
12.4 mM Sugar-beet Juice Molasses		
Before clinoptilolite treatment	105.90	52%
After clinoptilolite Treatment	51.26	

CHAPTER 5

MODELING

5.1 Cell Growth

The cell concentration change in the photobioreactor is divided into three main phases; lag phase, exponential phase and stationary phase. In the lag phase, after inoculation of the bacteria to the photobioreactor, the cells adjust to the new environment and synthesize enzymes, ready to begin reproducing. The duration of the lag phase depends on the inoculum's growth medium. In this study, the inoculum composition (20mM/10 mM Ac/Glu) is similar to the hydrogen production medium (40 mM/2 mM Ac/Glu) in that it contains the same carbon and nitrogen source (acetate and glutamate) in different concentrations. In the exponential phase, high cell growth is observed. The cell growth rate is proportional to the cell concentration. The cells divide at maximum rate, utilizing the available nutrients for growth. In this study, hydrogen production was observed in this phase. In the stationary phase, the cell growth stops due to the depletion of nutrients and essential metabolites. Generally, many fermentation products are produced in this phase. After the stationary phase, due to depletion of nutrients and other factors like formation of toxic or inhibitory by-products, the cell concentration decreases (Fogler, 2006). The stages in the cell growth are shown in Figure 5.1.

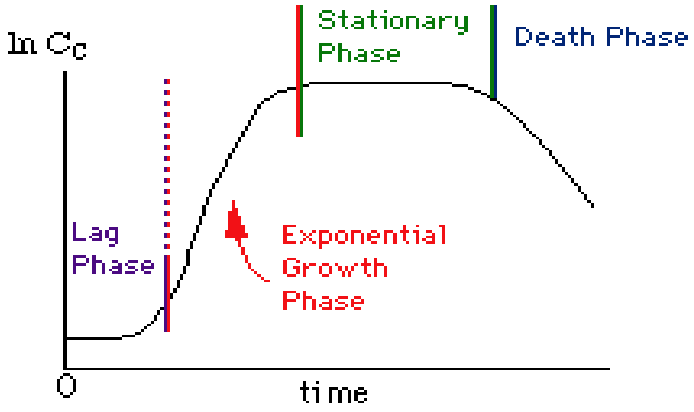


Figure 5.1 Phases of bacterial cell growth (Fogler, 2006).

Purple non-sulphur bacteria have been shown to retain their capacity to produce hydrogen in the stationary phase (Zürer *et al*., 1979, Sasikala *et al*., 1991, Koku *et al*., 2003, Melis *et al*., 2008). So for the commercial exploitation of continuous hydrogen production, a nearly constant hydrogen evolution can be achieved using fed-batch systems. In this system, the feed is replenished at consecutive times in the non-growing cells. This strategy is applied in this study.

The cell growth can be theoretically represented as:

$$\frac{dX}{dt} = \mu X \qquad (5.1)$$

where X is the cell dry weight concentration (gdcw/l_c) and μ is the specific growth rate (h^{-1}).

5.2 The Logistic Growth Model

The logistic model is a suitable method for representing the entire cell growth curve as it includes all the phases; the lag phase, exponential and stationary phases. The hydrogen production experiments done in this study were found to fit this model. The model can be shown as:

$$\mu = k_c\left(1-\frac{X}{X_{max}}\right) \tag{5.2}$$

where k_c is the apparent specific growth rate (h^{-1}), X is the cell dry weight concentration (gdcw/l_c) and X_{max} is the maximum cell dry weight concentration (gdcw/l_c). Substituting Equation 5.2 into Equation 5.1 and integrating, Equation (5.3) is obtained.

$$X = \frac{X_0 \exp(k_c t)}{\left(1-\frac{X_0}{X_{max}}\right)\left(1-\exp(k_c t)\right)} \tag{5.3}$$

where X_0 is the initial bacterial cell concentration.

The experimental data collected in this study were fitted into the logistic model using a program for fitting curves, Curve Expert 1.3. The results are shown in the following pages.

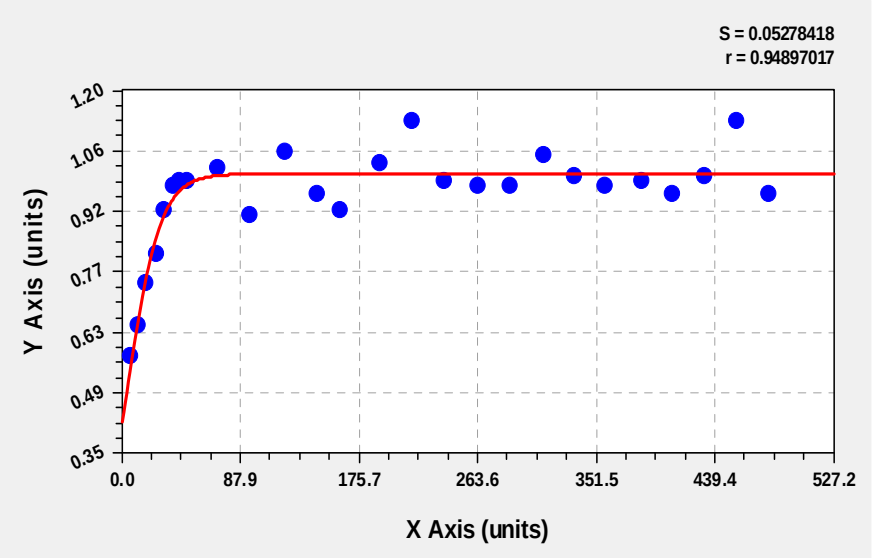


Figure 5.2 The logistic model for the *Rhodobacter capsulatus* (DSM 1710) CPBR outdoor experiment (13th September, 2008 – 3rd October, 2008). 40 mM / 2 mM Ac/Glu feed media was used in the experiment.

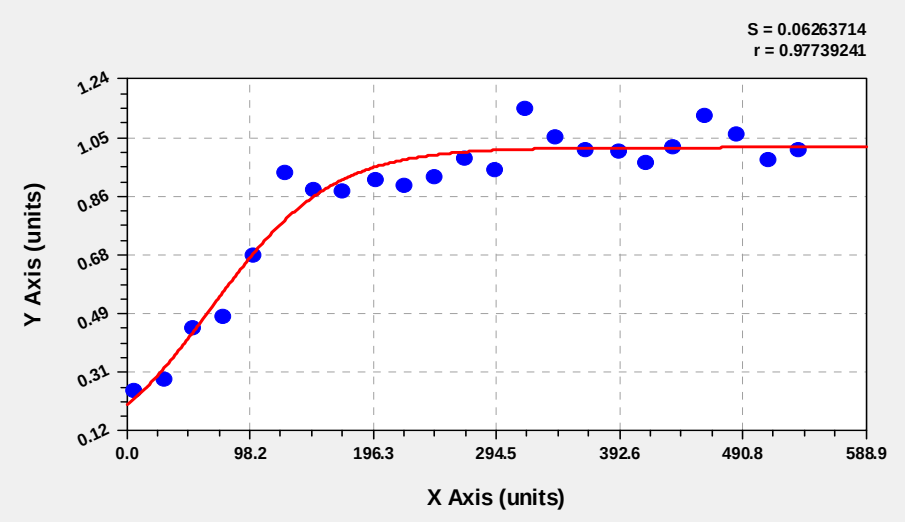


Figure 5.3 The logistic model for the heat adapted *Rhodobacter capsulatus* (DSM 1710) CPBR outdoor experiment (20th September, 2008 - 29th December, 2008). 40 mM/ 2 mM Ac/Glu feed media was used in the experiment.

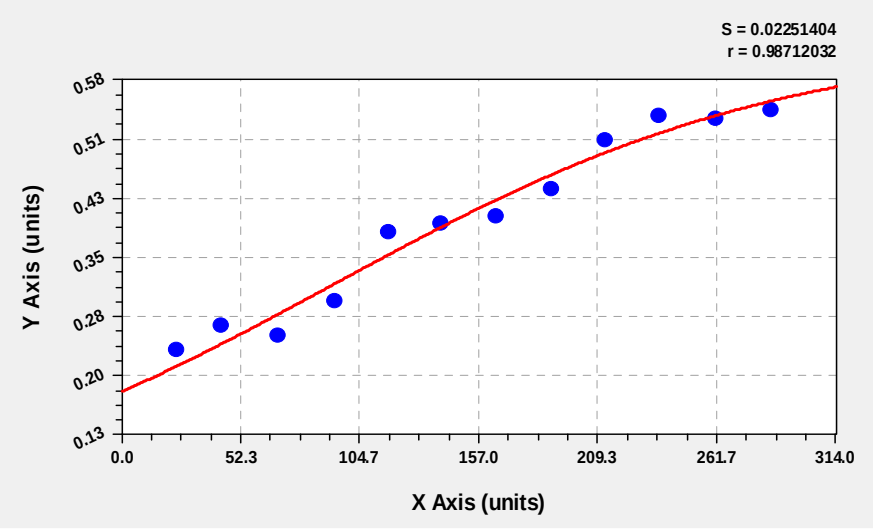


Figure 5.4 The logistic model for the *Rhodobacter capsulatus* YO3 (Hup^-) outdoor experiment (16th October – 24th December, 2008). 40 mM / 2 mM Ac/Glu feed media used in the experiment.

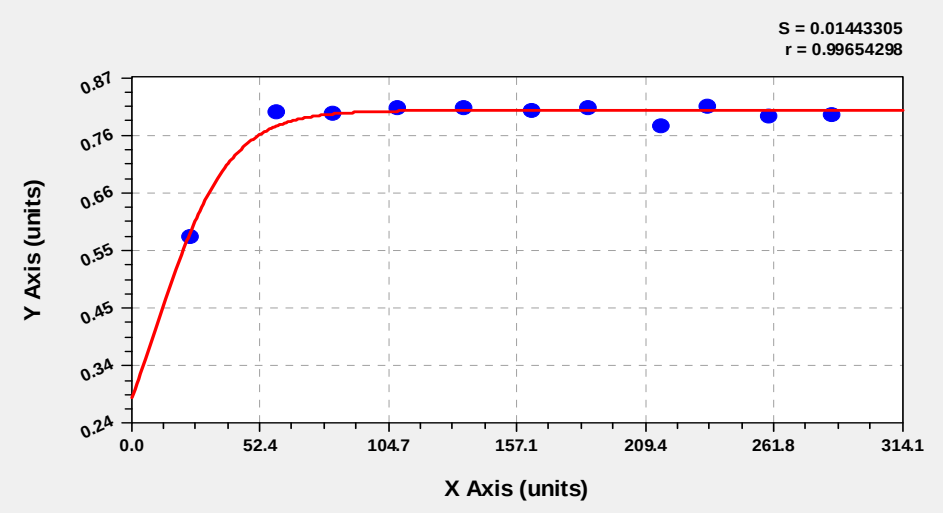


Figure 5.5 The logistic model for the *Rhodobacter capsulatus* YO3 (Hup$^-$) 60 mM/3 mM Ac/Glu fed CPBR indoor experiment (21st March – 3rd May, 2009).

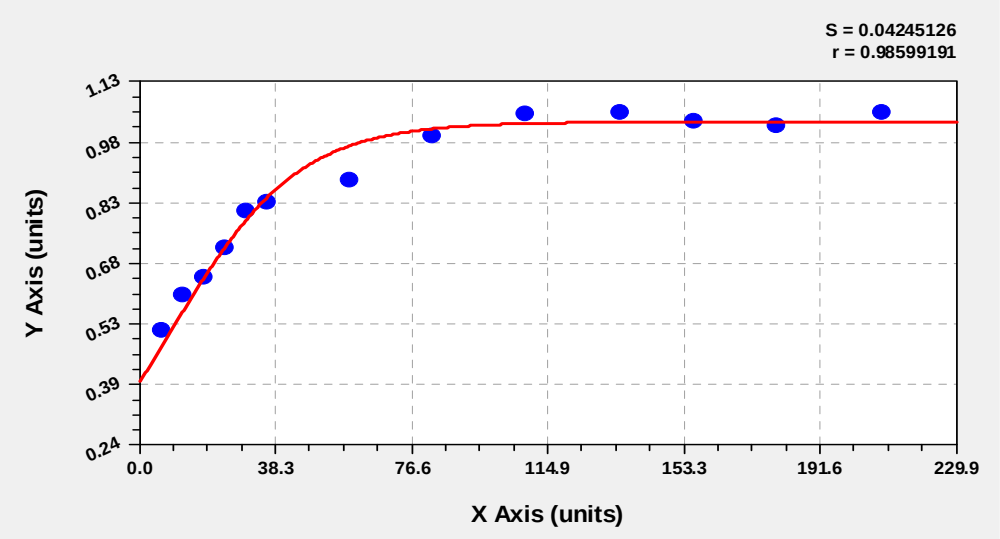


Figure 5.6 The logistic model for the *Rhodobacter capsulatus* YO3 (Hup$^-$) 80 mM/4 mM Ac/Glu CPBR indoor experiment (21st March – 1st April, 2009).

Table 5.1 Logistic model constant parameters for the continuous photobioreactors.

CPBR	*R.capsulatus* WT Outdoor Exp. (40mM/2 mM Ac/Glu)	Heat Adapted *R.capsulatus* WT Outdoor Exp. (40mM/2 mM Ac/Glu)	*R.capsulatus* YO3 (HUP^-) Outdoor Exp. (40 mM/2 mM Ac/Glu)	*R.capsulatus* YO3 Hup^- Indoor Exp. (60 mM/3 mM Ac/Glu)	*R.capsulatus* YO3 (Hup^-) (80 mM / 4 mM Ac/Glu)
X_{0e}	0.42	0.21	0.16	0.29	0.311
X_{0LM}	0.43	0.22	0.18	0.28	0.34
$(X_{max})_e$	1.42	1.01	0.54	0.81	1.05
X_{maxLM}	1.03	1.02	0.55	0.81	1.03
μ_{emax}	0.018	0.0046	0.0017	0.012	0.0017
k_c	0.018	0.0048	0.0015	0.012	0.0013

5.3 Discussion

The logistic model very well defines the biomass change with time in all the experiments. It indicates average biomass level that should be attained in stable operations. The increase in cell density depends on local or momental factors such as the increase in light intensity, temperature, acetate concentration and pH. The cell growth rate becomes zero when the cell concentration reaches the maximum biomass concentration. Since *R. capsulatus* is a photosynthetic bacteria, it can grow with illumination. So the change in the external effects may differently influence the maximum cell concentration, which is about 1 gdcw/l_c for the *R. capsulatus* (DSM 1710). The case where maximum cell concentration is 0.55 gdcw/l_c must be re-examined. This difference could most probably be because of the differences in light intensity. Bacteria has a threshold cell concentration above which the cells do not get enough energy from light and so stop growing. When the cell concentration reaches a minimum value, the cells are stimulated with light and start growing again.

These experiments concur with the findings with *R. sphaeroides* (Koku *et al*., 2003; Eroğlu, *et al*., 2008). They prove that the highest input of light energy is utilised for cell growth. The substrates carbon source and nitrogen source must be kept at an optimum level. Acetate is mainly utilized for hydrogen production and the by-products for storage purposes. The specific growth rate μ is in concurrence with k_c. This asserts the fit of the logistic model to the experimental data.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Several conclusions can be drawn from the indoor and outdoor experiments carried out in the continuous photobioreactors.

- Acetate is a suitable carbon source for continuous hydrogen production using *Rhodobacter capsulatus* in long term. It is utilized by the PNS bacteria for growth and hydrogen production.
- *Rhodobacter capsulatus* YO3 (Hup^-) strain is more suitable for hydrogen production on acetate than *Rhodobacter capsulatus* (DSM 1710) and its heat adapted strains.
- Hydrogen production levels depend on the photobioreactor irradiance level, temperature and C/N ratio of the feed medium.
- The optimum C/N ratio for *Rhodobacter capsulatus* YO3 (Hup^-) is 25. The feed media should contain 40 mM acetate and 4 mM glutamate.
- Stable biomass growth can be attained by adjusting the glutamate concentration (nitrogen source) in the feed medium.
- The presence of endogenous substrates leads to obtaining of over 100% substrate conversion efficiency by the PNS bacteria.
- Formic acid is formed as a fermentation end product when acetate is used as carbon source to the *Rhodobacter capsulatus* YO3 (Hup^-).
- Total gas produced in the continuous photobioreactors experiments using *Rhodobacter capsulatus* comprise 87-95% hydrogen with the rest being CO_2.

- pH range between 7-7.5 during hydrogen production in continuous photobioreactors using *Rhodobacter capsulatus*.

2. Several conclusions can be drawn from the ammonium ion removal experiments using clinoptilolite.

- Batch pre-treatment of clinoptilolite is an effective way of increasing the zeolite ion exchange capacity.
- Clinoptilolite zeolite is suitable to use in decreasing ammonium ion content in the dark fermenter effluent from thermophilic fermentation.

3. The logistic model fit the experimental data from the continuous photobioreactors.

6.2 Recommendations

1. Further experiments with the optimized feed composition can be done using the *Rhodobacter capsulatus* YO3 (Hup^-) strain in continuous panel photobioreactors. They include:

- An indoor experiment with higher light intensity ($270 W/m^2$) to investigate its effect on biomass, hydrogen production and formic acid formation through fermentation.
- Indoor experiments to investigate the effect of increasing feed composition while maintaining the optimized C/N ratio constant.
- An outdoor experiment with the optimized feed to test the culture performance under natural sunlight in outdoor conditions.

2. Test of the heat adapted *Rhodobacter capsulatus* (DSM 1710) in outdoor conditions in high temperatures and light intensity during the summer.

3. Design of a cooling system for the panel photobioreactors as operation in higher light intensities would lead to temperature increase.

4. Suggestions in the reduction of ammonium ion using clinoptilolite zeolite include:

- Use of different masses of clinoptilolite to determine the ion exchange capacities of the pretreated zeolite.
- Use of ammonium ion measuring kit in determining the ammonium ion content in the dark fermenter effluent. This would give more accurate results because ions like K^+ could interfere with the ammonium ion measurement electrode.

5. Suggestions on the modeling of the continuous photobioreactors are:

- To try fitting the logistic model to more experimental data from the continuous experiments.
- Re-examine factors which may affect the growth of cells in the photobioreactors and determine a relation between these parameters and the cell concentration. Parameters such as light intensity, temperature, cell concentration, pH and light and dark cycle, could be examined by performing different experiments. The relations found can be used to create a model that will depict the behavior of the cells in certain conditions.

REFERENCES

Abusafa, A., Yücel, H., 2002, ‘‘Removal of 137Cs from Aqueous Solutions Using Different Cationic Forms of a Natural Zeolite: Clioptilolite’’, Separation and Purification Technology, 28: 103 – 116.

Adam, P. S., Andrew, E. L., 2007, ‘‘Exergy Analysis of Hydrogen Production via Steam Methane Reforming’’, Int. Journal Hydrogen Energy, 32: 4811 – 4820.

Akkerman, I., Janssen, M., Rocha, J., Wijffels, R.H., 2002, “Photobiological hydrogen Production: Photochemical Efficiency and Bioreactor Design”, Int. J. Hydrogen Energy, 27: 1195 – 1208.

Arai, T., Wakayama, T., Okana, S., Kitamura, H., 1998, “Open Air Hydrogen Production by Photosynthetic Bacteria Used Solar Energy During Winter Seasons in Central Japan”, presented in Int. Conference on Biological Hydrogen Production, Hawaii, USA.

Arik, T., Ufuk G., Yücel, M., Türker L., Sediroğlu, V., Eroğlu, İ, ‘‘Photoproduction of hydrogen by *Rhodobacter sphaeroides* OU001’’, In: Proceedings of the 11th World Hydrogen Energy Conference, vol. 3. Stuttgart, Germany, 1996. p. 2417–24.

Asada, Y., Ohsawa, M., Nagai, Y., Ishimi, K., Fukatsua, M.., Hideno, A., Wakayama, T., Miyake, J., 2008, ‘‘Re-evaluation of Hydrogen Productivity from Acetate by some Photosynthetic Bacteria’’, Int. J. of Hydrogen Energy 33: 5147 – 5150

Aşiroğlu, S., 2006, “Lead (II) and Ammoniun Exchange on Na-Form of Gördes Clinoptilolite’’, MSc. Thesis in Chemical Engineering, Middle East Technical University, Ankara, Turkey.

Avcıoğlu, S.G., Androga, D.D, Uyar, B., Özgür, E., Gündüz, U., Yücel, M., Eroğlu, İ., 2009, ‘‘Continuous Hydrogen Production by *R. capsulatus* on Acetate in Panel Photobioreactors’’, HYPOTHESIS VIII, Lisbon, Portugal, April 1-3, 2009.

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

Barbosa, M.J., Rocha, J.M.S., Tramper J., Wijffels, R.H., 2001, ‘‘Acetate as a Carbon Source for Hydrogen Production by Photosynthetic Bacteria’’, J. Biotechnol., 85:25–33.

Bayraktaroğlu, 2006, “Multicomponent Ion Exchange on Clinoptilolite’’, MSc. Thesis in Chemical Engineering, Middle East Technical University, Ankara, Turkey.

Benemann, J.R., Berenson J.A., Kaplan N.O., Kauren M.D., 1973, ‘‘Hydrogen Evolution by a Chloroplast-Ferredoxin-Hydrogenase System’’, Proc. National Academy Science 70:2317-20.

Berktaş, B.M., Bircan A., 2003 ‘‘Effects of Atmospheric Sulphur Dioxide and Particulate Matter Concentrations on Emergency Room Admissions Due to Asthma in Ankara’’, Tuberculosis and Thorax Journal, 51: 231-238.

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

Biel, A.J., 1986, "Control of Bacteriochlorophyll Accumulation by Light in *Rhodobacter capsulatus*", Journal of Bacteriology, 168: 655-659.

Boran, E., Özgür, E., Gebicki, J., van der Burg, J., Yücel, M., Gündüz, U., Modigell, M., Eroğlu, İ., 2009, ''Investigation of Influencing Factors For Biological Hydrogen Production By *R. capsulatus* in Tubular Photo-bioreactors'', Presentation, 4th Joint Event PRES'09 & ICheaP-9, 10th -13th May 2009, Rome, Italy.

Bockris, J'O.M., 1981, ''The Economics of Hydrogen as a Fuel'', Int. J. Hydrogen Energy, 6,223-241.

Braunegg G., Sonnleitner B., Lafferty R.M., 1978, ''A Rapid Gas Chromatographic Method for the Determination of Boly-β-hydroxybutyric Acid in Microbial Biomass'', Biotechnology and Bioengineering, 6: 29-37.

Breck, D.W., 1971, " Zeolites Molecular Sieves, Structure, Chemistry and Use" pp 4-6, 128-130, 213, 231, John Wiley and Sons, Inc.,New York.

Burgess, G., Fernandez-Velasco, J.G., Lovegrove K., 2006, ''Materials, Geometry and Net Energy Ratio of Tubular Photobioreactors for Microalgal Hydrogen Production'', WHEC 16 / 13-16 June 2006 , Lyon, France.

Carlozzi, P., 2000, ''Hydrodynamic Aspects and Arthrospira Growth in Two Outdoor Tubular Undulating Row Photobioreactors'', Appl. Mirobiol. Biotechnol. 54,14–22.

Chen, SD., Sheu, DS, Chen, W.M., Lo, Y.C, Huang, T.I., Lin, C.Y., 2007, ‘‘Dark Hydrogen Fermentation from Hydrolyzed Starch Treated with Recombinant Amylase Originating from *Caldimonas taiwanensis*’’, Biotechnol. Prog. 23:1312–20.

Claassen P.A.M., Vrije T., 2006, ‘‘Non-thermal Production of Pure Hydrogen from Biomass: HYVOLUTION’’, Int. Journal of Hydrogen Energy, 31: 1416 – 1423.

Clayton, R.K., 1966, “Spectroscopic Analysis of Bacteriochlorophylls In-vitro and In-vivo”, Photochemistry and photobiology, 5: 669-677.

Cohen-Bazire, G., Sistrom W.R., Stanley, R.Y., 1957, ‘‘Kinetic Studies of Pigment Synthesis by Non-Purple Sulphur Bacteria’’, J.Cell. Comp. Physiol. 49: 25-68.

Collot, A-G., 2002., ‘‘Matching Gasifiers to Coals’’, IEA Clean Coal Centre, London, UK.

Czuppon, T.A., Knez S.A., Newsome, D.S., Othmer Encyclopedia of Chemical Technology, New York: Wiley 1996. p. 884.

Çulfaz, A., Yagız, M., 2004, ‘‘Ion Exchange Properties of Natural Clinoptilolite: Lead, Sodium and Cadmium-sodium Equilibria’’, Separation and Purification Technology, 37: 93 – 105.

Das, D., Veziroğlu T.N., 2001 “Hydrogen Production by Biological Processes: A Survey of Literature”, Int. J. Hydrogen Energy, 26: 13-28.

Eroglu, E., 2006, "Biological Hydrogen Production from Olive Mill Wastewater and its Applications to Bioremediation", Ph.D. Thesis in Chemical Engineering, Middle East Technical University, Ankara.

Eroğlu, I., Aslan, K., Gündüz, U., Yücel, M., Türker, L., 1999, ''Substrate Consumption Rates for Hydrogen Production by *Rhodobacter sphaeroides* in a Column Photobioreactor'', J. Biotechnol. 70: 103–113.

Eroğlu, I., Tabanoglu, A., Gündüz, U., Eroglu, E., Yücel, M., 2008, "Hydrogen Production by *Rhodobacter sphaeroides* O.U.001 in a Flat Plate Solar Bioreactor", Int. J. Hydrogen Energy,

Fascetti, E., and Todini, O., 1995, "*Rhodobacter sphaeroides* RV Cultivation and Hydrogen Production in One and Two-stage Chemostat", Appl. Microbioal. Biotechnology, 44: 300-305.

Fang, H.H.P., Liu, H., Zhang, T., 2002, ''Characterization of a Hydrogen Producing Granular Sludge'', Biotechnology, Bioengineering, 78: 44-52.

Fogler, H.S., 2006 ''Elements of Chemical Reaction Engineering'', Prentice Hall International Series, 4th Edition.

Fuller, R.C., Smillie, R.M., Sisler, E.C., Kornberg, H.L., 1961, "Carbon Metabolism in *Chromatium*", J. Biol. Chem., 236: 2140.

Gaffron ,H., Rubin, J., 1992, '' Fermentative and Photochemical Production of Hydrogen by Algae'', J. Gen. Physiology, 26: 219-40.

Gebicki, J., Modigell, M., Schumacher M., ‘‘Continuously Operated Photobioreactor for Hydrogen Production’’, R.W.T.H. HYVOLUTION POSTER, Process Net Jahrestagung, 2007, 16-18 October 2007, Aachen, Germany.

Gogotov, I.N., 1986, ‘‘Hydrogenases of Phototrophic Microorganisms’’, *Biochimie,* 68:181–187.

Groenestijn, J.W., Hazewinkel, J.H.O., Nienoord, M., Bussmann, P.J.T., 2002, ‘‘Energy aspects of Biological Hydrogen Production in High Rate Bioreactors Operated in the Thermophilic Temperature Range’’, Int. J. Hydrogen Energy 27:1141–1147.

Groenestijn J.W., Geelhoed J.S., Goorissen H.P., Meesters K.P.M., Stams A.J.M., Claassen P.A.M.. 2009., ‘‘Performance and Population Analysis of a Non-Sterile Trickle Bed Reactor Inoculated with *Caldicellulosiruptor saccharolyticus*, a Thermophilic Hydrogen Producer’’, Biotechnol., 102:1361-1367.

Hall, D.O., Markov, S.A., Watanable, Y., Rao, K.K., 1995., ‘‘The Potential Applications of Cyanobacterial Photosynthesis for Clean Technologies’’, Photosynth. Res. 46, 159–167.

Hallenbeck, P.C., Benemann, J.R., 2002, “Biological Hydrogen Production: Fundamentals and Limiting Processes”, Int. J. of Hydrogen Energy, 27: 1185 –1193.

Hassmann, K., Kuhne, H. M., 1993, ‘‘Primary Energy Sources for Hydrogen Production’’, Int. J. Hydrogen Energy, 18: 635-640.

Hillmer, P., Gest, H., 1977, "Hydrogen metabolism in the Photosynthetic Bacterium *Rhodopseudomonas capsulata*: Hydrogen Production by Growing Cultures", J. Bacteriol.,129: 724-731.

Hoekema, S., Bijmans, M., Janssen, M., Tramper, J., Wijffels, R.H., 2002, "A Pneumatically Agitated Flat-Panel Photobioreactor with Gas Re-circulation: Anaerobic Photoheterotrophic Cultivation of a Purple Non-sulfur Bacterium", Int. J. Hydrogen Energy, 27: 1331– 1338.

Hirabayashi, H., Amakawa, M., Kamimura, Y., Shino Y., Satoh, H., Itoh, S., Tamiaki, H., 2006, "Analysis of Photooxidized Pigments in Water Soluble Chlorophyll Protein Complex Isolated from Chenopodium Album", Journal of Photochemistry and Photobiology, 183: 121–125.

Imhoff, J.F., Truper H.G., Pfennig, N., 1984, ''Rearrangement of the Species and Genera of the Phototrophic Purple Non-Sulfur Bacteria'', Int. J. Syst. Bacteriol. 34: 340–343.

Jouanneau,Y., Wong, B., Vignais, P.M., 1985, ''Stimulation by Light of Nitrogenase Synthesis in Cells of *Rhodopseudomonas capsulata* Growing in N-limited Continuous Cultures'', Biochimica. Biophys. Act., 808, pp.149-155

Kadar, Z., Vrije T., Budde, M.A.W., Szengyel, Z., Reczy, K., Claassen, P.A.M., 2003, ''Hydrogen Production from Paper Sludge Hydrolysate'', Appl. Biochem. Biotechnol. 105–108:557–566.

Kadar, Z., Vrije T., van Noorden G.E., Budde, M.A.W., Szengyel, Z., Reczy, K., Claassen, P.A.M., 2004, ''Yields from Glucose, Xylose, and Paper Sludge Hydrolysate During Hydrogen Production by the Extreme Thermophile *Caldicellulosiruptor saccharolyticus*'', Appl. Biochem. Biotechnol. 113–116:497–508.

Kengen, S.W.M., Goorissen, H.P., Verhaart, M., Stams, A.J.M., van Niel, E.W.J., Claassen, PAM., 2008, ‘‘Biological Hydrogen Production by Anaerobic Microorganisms’’, Chichester: John Wiley & Sons. p 197–221.

Khatipov, E., Miyake, M., Miyake, J., Asada, Y., 1998, “Accumulation of Poly-hydroxybutyrate by *Rhodobacter sphaeroides* on Various Carbon and Nitrogen Substrates”, FEMS Microbiology Letters, 162: 39-45.

Kim, J.S., Ito, K., Takahashi, H., 1980, “The Relationship Between Nitrogenase Activity and Hydrogen Evolution in *Rhodopseudomonas palustris*”, Agric. Biol.Chem, 44: 827–833.

Kleerebezem, R., van Loosdrecht, M.C.M., 2007, ‘‘Mixed Culture Biotechnology for Bioenergy Production’’, Current Opinion in Biotechnology 18:207–212.

Koku H., 2001, “Hydrogen Metabolism and Factors Affecting Hydrogen Production in *Rhodobacter sphaeroides*”, MSc. Thesis in Chemical Engineering, Middle East Technical University, Ankara, Turkey.

Koku, H., Eroğlu, I., Gunduz, U., Yucel, M., Turker, L., 2002, ‘‘Aspects of the Metabolism of Hydrogen Production by *Rhodobacter sphaeroides*’’, Int. J. Hydrogen Energy, 2:1325-1329.

Koku, H., Eroğlu, İ., Gündüz, U., Yücel, M., Türker, L., ‘‘Kinetics of Biological Hydrogen Production by *Rhodobacter sphaeroides* OU 001’’, Int. J. Hydrogen Energy 2003; 28 :381–8.

Krahn, E., Schneider, K., Muller, A., 1996, “Comparative Characterization of Hydrogen Production by the Conventional Mo Nitrogenase and the Alternative "Iron-Only" Nitrogenase of *Rhodobacter capsulatus* Hup$^-$ Mutants”, Appl. Biochem. Biotechnol., 70: 301-310.

Inglezakis, V.J., Hadjiandreu, K.J., Loizidou, M.D., Grigoropoulou, H.P., 2001, ‘‘Pretreatment of Natural Clinoptilolite in a Laboratory-scale Ion Exchange Packed Bed’’, Water Research, 35: 2161 – 2166.

Inglezakis, V.J., Grigoropoulou, H.P., 2004, ‘‘Effects of Operating Conditions on the Removal of Heavy Metals by Zeolite in Fixed Bed Reactors’’, Journal of Hazardous Materials, 112: 37 – 43.

Liu, B., Nan-Qi R., De-Feng, X., Jie, D., Guo-Xiang, Z., Wan-Qian, G., Ji-Fei, X., Guo-Jun, X., 2008, ‘‘Hydrogen Production by Immobilized *R. faecalis* RLD-53 using Soluble Metabolites from Ethanol Fermentation Bacteria *E. harbinense* B49,’’ Bioresource Technology 100, 2719–2723.

Mark, D. R., Marion P-B., and Lynne E. M., 2008, ‘‘Integrating Dark and Light Bio-hydrogen Production Strategies: Towards the Hydrogen Economy,’’ Reviews in Environmental Science and Biotechnology, 8: 2.

Mc, Auliffe, C. A., ‘‘Hydrogen and Energy’’, The Macmillan Press, London, 1980.

Melis, A., 2002, ‘‘Green Alga Hydrogen Production: Progress, Challenges and Prospects”, Int. J. Hydrogen Energy, 27: 1217 – 1228.

Meng, Ni., Dennis, Y.C. L., Michael K.H. L., Sumathy K., 2006, ‘‘An Overview of Hydrogen Production from Biomass’’, Int. Journal of Coal Geology, 65: 191– 212.

Meyer, J., Kelley B.C., Vignais, P.M., 1978, ‘‘Nitrogen Fixation and Hydrogen Metabolismin Photosynthetic Bacteria’’, Biochimie, 60: 245-260.

Miyake, J., Kawamura, S., 1987, ‘‘Efficiency of Light Energy Conversion to Hydrogen by Photosynthetic Bacteria *Rhodobacter sphaeroides*’’, Int. J. Hydrogen Energy, 12:147-9.

Miyake, J., 1989. ‘‘The Science of Biohydrogen’’, Biohydrogen, Editor: O.R. Zaborsky, Plenum Press, New York, pp 7-19.

Modigell, M., Holle, N., 1998, “Reactor Development for a Biosolar Hydrogen Production Process”, Renewable Energy, 14: 421-426.

Moralı, N., 2006, “Investigation of Zinc and Lead Removal from Aqueous Solutions Using Clinoptilolite’’, MSc. Thesis in Environmental Engineering, Middle East Technical University, Ankara,Turkey.

Mumpton, F.A., Sand, L.B., Natural Zeolites Occurrence Properties and Use, Pergamon Press, New York, 1978.

Nakada, E., Asada, Y., Arai, T., Miyake, J., 1995, ‘‘Light Penetration into Cell Suspensions of Photosynthetic Bacteria and Relation to Hydrogen Production’’, J. Ferment. Bioeng., 80:57.

Nakada, E., Nishikata, S., Asada, Y., Miyake, J., 1998, “Light Penetration and Wavelength Effect on Photosynthetic Bacteria Culture for Hydrogen Production.” In: Biohydrogen, Zaborsky OR, editor. New York: Plenum Press.

Niel, E.W.J., Budde, M.A.W., de Haas G.G., van der Wal F.J., Claassen P.A.M., Stams A.J.M., 2002, ‘‘Distinctive Properties of High Hydrogen Producing Extreme Thermophiles, *Caldicellulosiruptor saccharolyticus* and *Thermotogaelfii*’’, Int. J. Hydrogen Energy 27:1391–1398.

Ogbanna, J,C., Yada, H., Tanaka, H., 1995, ‘‘Light Supply Coefficient: A New Engineering Parameter for Photobioreactor Design’’, J. Ferment Bioeng., 80:369-376.

Ogbonna, J.C., Toshihiko, S., Tanaka, H., 1999, “An Integrated Solar and Artificial Light System for Internal Illumination of Photobioreactors”, Journal of Biotechnology, 70: 289–297.

Ooshima. H., Takakuwa, S., Katsuda, T., Okuda, M., Shirasawa, T., Azuma, M., Kato, J., 1998, ‘‘Production of Hydrogen by a Hydrogenase-defcient Mutant of *Rhodobacter capsulatus*’’, J. Ferment Bioeng., 85: 470–4.

Ormerod, J. G., Ormerod K.S., Gest, H., 1961, ‘‘Light dependent Utilization of Organic Compounds and Photoproduction of Molecular Hydrogen by Photosynthetic Bacteria: Relationships with Nitrogen Metabolism”, Arch. Biochem. Biophys. 94: 449-463.

Ouki S.K., Cheeseman C., Perry R., 1994, ‘‘Natural Zeolite Utilisation in Pollution Control: A Review of Applications to Metal Effluents’’, Journal of Chemical Technology and Biotechnology, 59: 121 – 126.

Ouki S.K., Kavannagh M., 1999, ‘‘Treatment of Metals-contaminated Wastewaters by use of Natural Zeolites’’, Water Science and Technology, 39: 115 – 122.

Özgür, E., Uyar, B., Öztürk, Y., Yücel, M., Gündüz, U., Eroğlu, İ., 2009, ‘‘Biohydrogen Production by *Rhodobacter capsulatus* on Acetate at Fluctuating Temperatures”, Resources, Conservation and Recyling, 2147, 5.

Öztürk, Y., 2005, “Characterisation of the Genetically Modified Cytochrome Systems and Their Application to Biohydrogen Production in *Rhodobacter Capsulatus*”, Ph. D. Thesis in Biotechnology, Middle East Technical University, Ankara.

Öztürk Y., Yücel M., Daldal F., Mandacı S., Gündüz U., Türker L., Eroglu I., 2006, ‘‘Hydrogen Production by using *Rhodobacter capsulatus* Mutants with Genetically Modified Electron Transfer Chains’’, Int. J. Hydrogen Energy, 31:1545–52.

Pellerin, N.B., and Gest, H., 1983, “Diagnostic Features of the Photosynthetic Bacterium *Rhodopseudomonas sphaeroides*”, Current Microbiology, 9: 339-344.

Rainey, F.A., Donnison, A.M., Janssen, P.H., Saul, D., Rodrigo, A., Bergquist, P.L., Daniel, R.M., Stackebrandt, E., Morgan, H.W., 1994, ‘‘Description of *Caldicellulosiruptor saccharolyticus* gen. nov., sp. nov. : An Obligately Anaerobic, Extremely Thermophilic, Cellulolytic Bacterium’’, FEMS Microbiol. Lett. 120:263–266.

Ramachandran, R., Menon, R.K., 1998, ‘‘An Overview of Industrial Uses of Hydrogen’’, Int. J. Hydrogen Energy, 23:593-8.

Reith, J.H, Wijffels R.H., Barten, H., Bio-methane and Bio-hydrogen: Status and Perspectives of Biological Methane and Hydrogen Production. The Hague: Smiet offset; 2003. p. 103–23.

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.

Richmond, A., Cheng-Wu, Z., 2001, “Optimization of a Flat Plate Glass Reactor for Mass Production of *Nannochloropsis sp*.Outdoors", J. Biotechnology, 85: 259-269.

Sarıoglu M., 2005, ‘‘Removal of Ammonium from Municipal Wastewater Using Natural Turkish (Dogantepe) Zeolite’’, Separation and Purification Technology, 41: 1- 11.

Sasaki, K., 1998, ‘‘Hydrogen and 5-aminolevulinic acid production by Photosynthetic Bacteria’’, In: Zaborsky, O.R.(Ed.), Biohydrogen. Plenum Press, London, pp. 133–142.

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”, Int. J. Hydrogen Energy, 15: 795-797.

Sasikala, K., Ramana, C.H.V., Rao, P.R., 1991. Environmental Regulation for Optimal Biomass Yield and Photoproduction of Hydrogen by *Rhodobacter sphaeroides* O.U.001’’, Int. J. 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 Microbiolog., 38:211-295

Sasikala, C., Ramana, C.V., Rao, R., 1997, ‘‘Environmental Regulation for Optimal Biomass Yield and Photoproduction of Hydrogen by *Rhodobacter sphaeroides* O.U. 001’’, Int. J. Hydrogen Energy, 16 : 597–601.

Segers, L., Verstrynge, L., Verstraete, W., 1981, ‘‘Product Patterns of Non-axenic Sucrose Fermentation as a Function of pH’’, Biotechnol. Lett. 3, 11: 635–640.

Segers, L., Verstraete, W., 1983, “Conversion of organic acids to Hydrogen by *Rhodospirillaceae* Grown with Glutamate or Dinitrogen as Nitrogen Source”, Biotechnol. Bioeng., 25: 2843–2853.

Semmens M.J., Seyfarth M., Sand L.B., Mumpton F.A., ‘‘Natural Zeolites, Occurence, Properties and Use’’, Pergamon Press, New York, 1978.

Shi, X.Y., Yu, H.Q., 2006, ‘‘ Continuous Production of Hydrogen from Mixed Volatile Fatty Acids with *Rhodopseudomonas capsulatus*’’, Int. J., Hydrogen Energy 2006, 31:1641–7.

Simpson, A.P., Lutz, A.E., 2007, ‘‘Energy Analysis of Hydrogen Production via Steam Methane Reforming’’, Int. J Hydrogen Energy, 32:4811–20.

Smith, G.D, Ewart, G.D., Tucker, W., 1992, ‘‘Hydrogen Production by Cyanobacteria’’, Int. J. Hydrogen Energy, 17: 695-8.

Stevens, P., Plovie, N., de Vos, P., de Ley, J., 1986, ‘‘Photoproduction of Molecular Hydrogen by *Rhodobacter sufidophilus*’’, System. Appl. Microbiol. 8: 19-23.

Senior, P.J., Dawes, E.A., 1973, ‘‘ The Regulation of Poly-b-Hydroxybutyrate Metabolism in Azotobacter beijerinckii’’, Biochem. J. 134: 225–0.

Suludere, D., 2001, “Investigation of Polyhydroxybutyrate (PHB) Production by Photosynthetic Bacterium: *Rhodobacter sphaeroides* O.U.001”, M.Sc. Thesis in Biology, Middle East Technical University, Ankara, Turkey.

Suzuki, Y., 1982, ‘‘On Hydrogen as a Fuel Gas’’, Int. J. Hydrogen Energy, 7: 227-230.

Szostak R., 1984 “Handbook of Molecular Sieves”, pp. 126-132, Van Nostrand Reinhold, New York.

Tabanoğlu, 2002, “Hydrogen Production by *Rhodobacter sphaeroides* O.U.001 in a Solar Bioreactor”, Msc. Thesis in Biotechnology, Middle East Technical University, Ankara, Turkey.

Tao, Y., Chen, Y., Wu, Y., He, Y., Zhou, Z., 2007, “High hydrogen Yield from a Two-Step Process of Dark and Photofermentation of Sucrose”, Int. J. Hydrogen Energy, 32: 200-206.

Tredici, M.R., 1999, ‘‘Bioreactors’’, In: Flickinger, M.C., Drew, S.W. (Editorss.), Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation, vol. 1. Wiley, New York, pp. 395–419.

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, pg.117-130.

Tredici, M.R., 2004, “Mass Production of Microalgae: Photobioreactors”. In ‘Handbook of Microalgal Culture, Biotechnology and Applied Phycology”, A. Richmond (Ed.), Blackwell Scientific, 178-214.

Trosehel, D., Müller, M., 1990, ‘‘Development of a Cell-free System to Study the Membrane Assembly of Photosynthetic Proteins of *Rhodobacter capsulatus*’’, Biochemisches Institut der Universitat, D-7800 Freiburg, Federal Republic of Germany.

Tsygankov, A.A., Hirata, Y., Miyake, M., Asada, Y., Miyake, J., 1993, ‘‘Hydrogen Evolution Photosynthetic Bacterium *Rhodobacter sphaeroides* RV Immobilized on Porous Glass’’, New Energy Systems and Conversions, Universal Academy Press, pp. 229–233.

Türkarslan, S., Yiğit, D.Ö., Aslan, K.., I. Eroğlu and Gündüz U., 1998, “Photobiological Hydrogen Production by *Rhodobacter sphaeroides* O.U. 001 by Utilization of Waste Water from Milk Industry”, In: O.R. Zaborsku, Editor, Biohydrogen, Plenum Press, New York.

UN Department of Economic and Social Affairs, Population Division, ‘‘ Press Release, 2009’’, http://www.un.org/esa/population/, 11th March, 2009.

Uyar, B., Gündüz, U., Yücel, M., Türker, L., Eroglu, I., 2007, “Effect of Light Intensity, Wavelength and Illumination Protocol on Hydrogen Production in Photobioreactors”, Int. J. Hydrogen Energy, 32: 4670– 4677.

Uyar, B., 2008, “Hydrogen Production by Microorganisms on Solar Bioreactors”, P.h.D. Thesis in Biotechnology, Middle East Technical University, Ankara, Turkey.

Veziroğlu, T.N., 1995, ‘‘Twenty Years of the Hydrogen Movement 1974 - 1994’’, Int. J. Hydrogen Energy, 20: 1-7.

Vignais, P.M., Colbeau, A., Willison, J.C., Jouanneau, Y., 1985, "Hydrogenase, Nitrogenase, and Hydrogen Metabolism in Photosynthetic Bacteria", Adv. Microbial Phys., 26: 154-234.

Vrati, S., 1984, ''Single Cell Protein Production by Photosynthetic Bacteria Grown on the Clarified Effluents of Biogas Plant'', Appl. Microbiol. Biotechnol. 19, 199-202

Vrije, T., Claassen, P.A.M., Dark Hydrogen Fermentations. In: Reith, J.H., Wijffels, R.H., Barten, H., (Editors), ''Bio-methane and Bio-hydrogen- Status and Perspectives of Biological Methane and Hydrogen Production'', the Hague: Smiet offset; 2003. p. 103–23.

Wakayama, T., Miyake, J., Toriyama, A., Kawasugi, T., Arai, T., Asada, Y., 1998, ''Photohydrogen Production Using Photosynthetic Bacterium *Rhodobacter sphaeroides* RV; Simulation of the Light Cycle of Natural Sunlight Using an Artificial Source'', In: Zaborsky OR. (Editor) Biohydrogen. London: Plenum Press.

Willison, J.C., 1988, "Pyruvate and Acetate Metabolism in the Photosynthetic Bacterium *R. capsulatus*", J. Gen. Microbiology, 134: 2429-2439.

Yiğit, D.Ö., Gündüz, U., Türker, L., Yücel, M., Eroglu I., 1999, "Identification of By-products in Hydrogen Producing Bacteria: *Rhodobacter sphaeroides* O.U. 001 Grown in the Wastewater of a Sugar Refinery", J. Biotechnol., 70: 125-131.

Zabut, B., Kahlout, K. E., Yücel, M., 2006, ''Hydrogen Gas Production by Combined Systems of *Rhodobacter sphaeroides* O. U. 001 and *Halobacterium salinarum* in a Photobioreactor'', Int. J. of Hydrogen Energy, 31: 1553- 1562.

Zhang, K., Kurano, N., Miyachi, S., 1999, "Outdoor Culture of a Cyanobacterium with a vertical Flat-plate Photobioreactor: Effects on Productivity of the Reactor Orientation, Distance Setting between the Plates and Culture Temperature", Appl. Microbiol. Biotechnol., 52: 781-786.

Zhang, H.S., Bruns M.A., Logan B.E., 2006, ''Biological Hydrogen Production by *Clostridium acetobutylicum* in an Unsaturated Flow Reactor'', Water Res. 40:728–734

Zorin, N.A., 1986, ''Redox Properties and Active Center of Phototrophic Bacteria Hydrogenases'', Biochimie 1986;68:97–101.

APPENDIX A

COMPOSITION OF THE FEED MEDIUM

Table A.1 The composition of the standard basal, growth and hydrogen production medium per litre of solution.

Component	Amount
Standard Basal Medium	
KH_2PO_4 (22 mM)	0.5g
$MgSO_4.7H_20$	0.5g
$CaCl_2.2H_2O$	0.5g
Vitamin Solution	1 ml
Fe-Citrate*	0.1 ml
Trace Elements	0.1 ml
Growth Medium**	
Acetate	20 mM
Glutamate	10 mM
Hydrogen Production Medium**	
Acetate	40 - 80 mM
Glutamate	2 - 4 mM

***** 0.5 g Fe-citrate was dissolved in 100 ml distilled water and sterilized by autoclaving.
******Consists of the standard basal medium plus acetate and glutamate.

Table A.2 The composition of trace element solution.

Composition Amount	**Amount**
HCl (25% v/v)	1 ml
$ZnCl_2$	70 mg
$MnCl_2 \times 4H_2O$	100 mg
H_3BO_3	60 mg
$CoCl_2 \times 6\ H_2O$	200 mg
$CuCl_2 \times 2\ H_2O$	20 mg
$NiCl_2 \times 6\ H_2O$	20 mg
$NaMoO_4 \times 2\ H_2O$	40 mg
H_2O	Complete to 1l

Table A.3 The composition of the vitamin solution.

Composition	**Amount**
Thiamine	500 mg
Niacin (Nicotinate)	500 mg
Biotin	15 mg
H_2O	Complete to 1 l

APPENDIX B

DETERMINATION OF THE ACETATE AND GLUTAMATE CONCENTRATION IN THE GROWTH MEDIUM

Equation B1 is the formation of *Rhodobacter capsulatus* biomass from acetic acid.

$$a\mathrm{CH_3COOH} + b\mathrm{C_5H_8NO_4.H_2O} \rightarrow c\mathrm{CH_{1.76}O_{0.38}N_{0.14}} + d\mathrm{CO_2} + e\mathrm{H_2O} \quad \text{(B1)}$$

acetic acid glutamate biomass

- Elemental balance

  C: $2a + 5b = c + d$ (B2)

  H: $4a + 10b = 1.76c + 2e$ (B3)

  O: $2a + 5b = 0.38c + 2d + e$ (B4)

  N: $b = 0.14c$ (B5)

  Unknowns variables: *a, b c, d, e*

  Equations: 4

We need to define one variable to solve the four elemental balance equations.

- Assuming $b = 0.14$

  $$c = \frac{0.14}{0.14} = 1$$

- Substituting Equations (B3) and (B4)

$$\frac{4a+10b-1.76c}{2} = 2a+5b-0.38c-2d$$

$$2a+5b-0.88c = 2a+5b-0.38c-2d$$

$$c = 4d \qquad \text{(B6)}$$

- From Equation (B6),

$$d = \frac{c}{4} = \frac{1}{4} = 0.25$$

- The values of the variables *b, c* and *d* obtained are substituted into Equation (B2).

$$2a+5b = c+d$$

$$a = \frac{c+d-5b}{2}$$

$$a = \frac{1+0.25-5(0.14)}{2}$$

$$a = 0.275$$

- The obtained values of variables *a, b, c* and *d* are substituted into Equation (B3).

$$4a+10b = 1.76c+2e$$

$$e = \frac{4a+10b-1.76c}{2}$$

$$e = \frac{4(0.275)+10(0.14)-1.76(1)}{2}$$

$$e = 0.37$$

- Inserting the obtained values of variables *a, b, c, d* and *e* into equation 1.

$$1.96\ CH_3COOH + C_5H_8NO_4.H_2O \rightarrow 7.14CH_{1.76}O_{0.38}N_{0.14} + 1.79CO_2 + 2.64\ H_2O \quad \text{(B7)}$$

- From the elementary Equation (B2), the feed C/N is determined as:

$$\frac{C}{N} = \frac{1.96 \times 2 + 1 \times 5}{1} = 9$$

- The growth medium C/N ration is determined as:

20 mM/10 mM Ac/Glu growth medium $\frac{C}{N} = \frac{20 \times 2 + 10 \times 5}{10} = 9$

APPENDIX C

OPTICAL DENSITY-DRY WEIGHT CALIBRATION CURVES

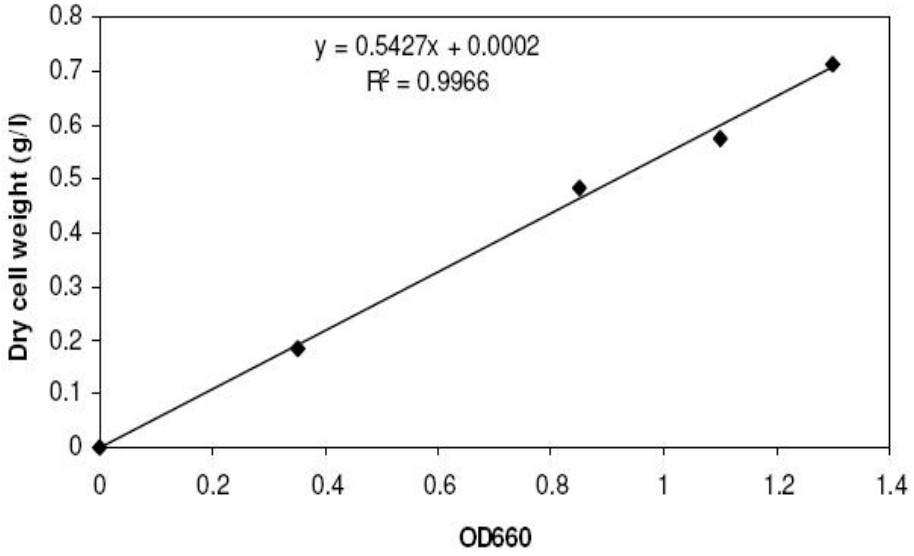


Figure C.1 Calibration curve and the regression trend line for *Rhodobacter capsulatus* (DSM 1710) dry weight versus OD660 (Uyar, 2008).

- An optical density of 1.0 at 660nm corresponds to a cell density of 0.54 gram dry cell weight/liter of culture of *Rhodobacter capsulatus* (DSM 1710).

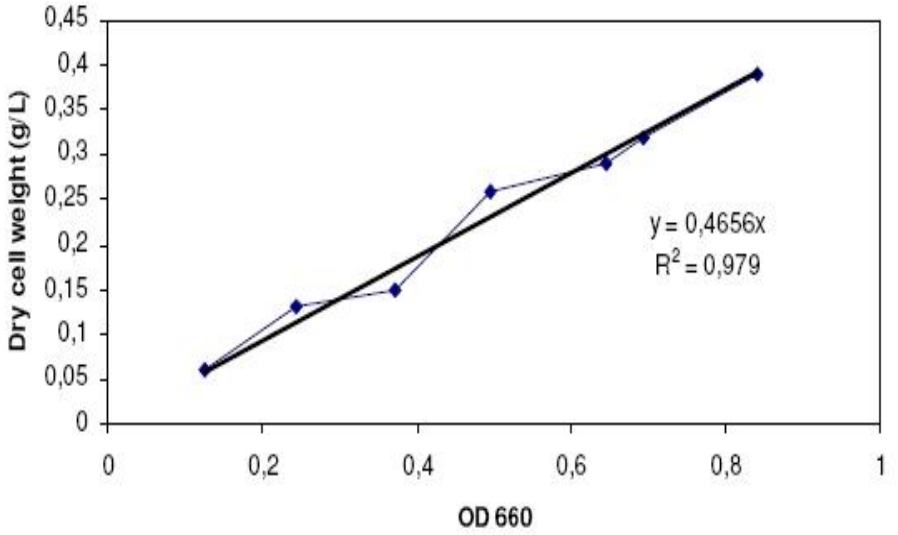


Figure C.2 Calibration curve and the regression trend line for *Rhodobacter capsulatus* YO3 dry weight versus OD660 (Öztürk, 2005).

- An optical density of 1.0 at 660nm corresponds to a cell density of 0.47 gram dry cell weight/liter of culture of *Rhodobacter capsulatus* YO3 (Hup^-).

APPENDIX D

Rhodobacter capsulatus LIGHT ABSORBTION SPECTRA

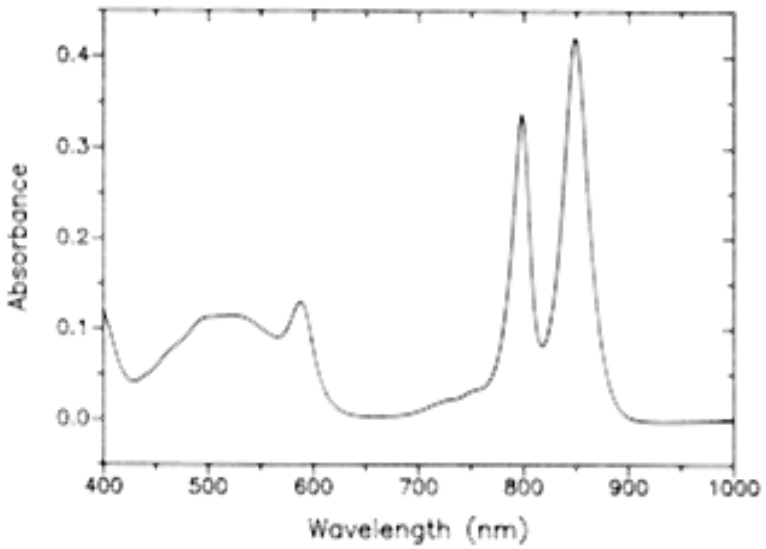
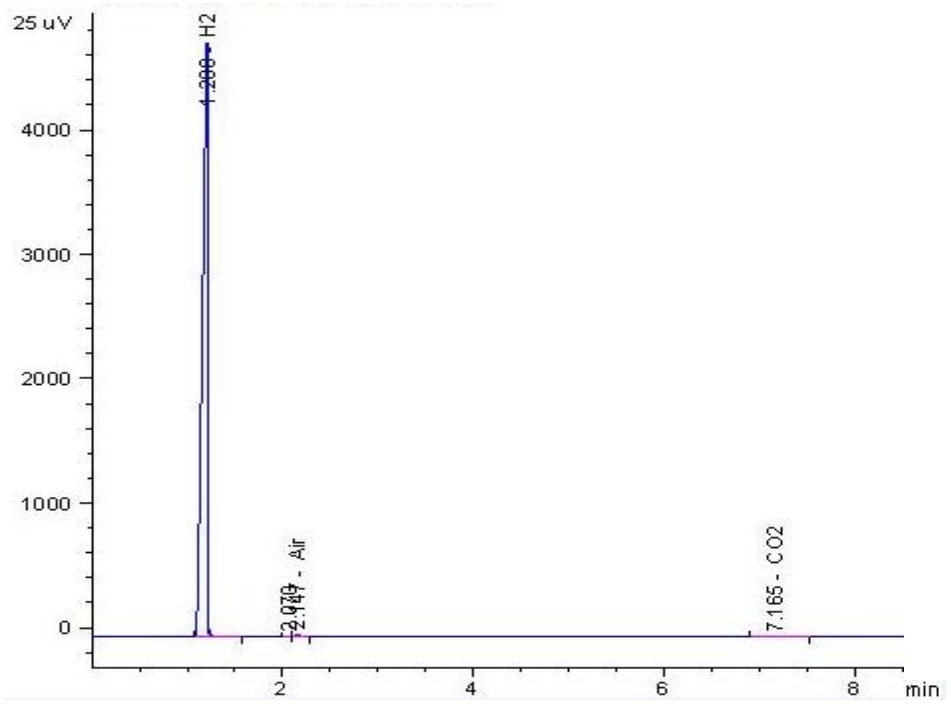


Figure D The light absorption spectrum of *Rhodobacter capsulatus* (Trosehel and Müller, 1990).

APPENDIX E

SAMPLE GAS CHROMATOGRAM



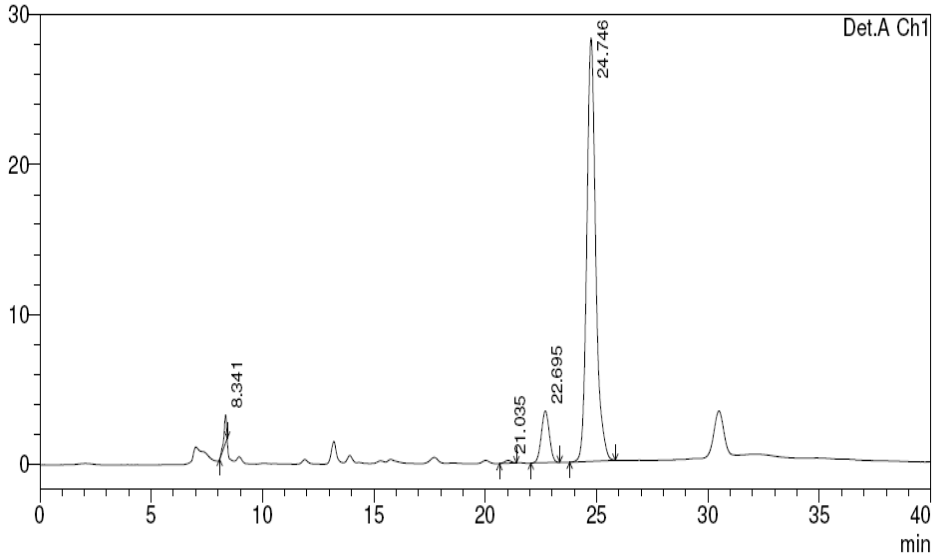
RetTime [min]	Type	Area [25 uV*s]	Amt/Area	Norm %	Grp	Name
1.208	BB	1.78157e4	5.36760e-3	92.790983		H2
2.147	VB	104.16668	4.30476e-2	4.351104		Air
7.165	BB	52.88443	5.56929e-2	2.857913		CO2
Totals :				100.000000		

Figure E Sample gas analysis chromatogram (analysis done by Agilent Technologies 6890N gas chromatography).

APPENDIX F

ORGANIC ACID ANALYSIS

F1. Sample organic acid HPLC chromatogram



PeakTable

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.341	14689	1859	1.654	5.516
2	21.035	3735	182	0.420	0.542
3	22.695	86422	3453	9.730	10.248
4	24.746	783359	28200	88.196	83.694
Total		888205	33695	100.000	100.000

Figure F.1 Sample HPLC analysis chromatogram (concentrations measured by Shimadzu 10A series high performance liquid chromatography). Peak 1 (mobile phase- H_2SO_4), Peak 2 (lactic acid), Peak 3 (Formic acid) and Peak 4 (acetic acid).

F2. Sample Acetic Acid HPLC Calibration Curve

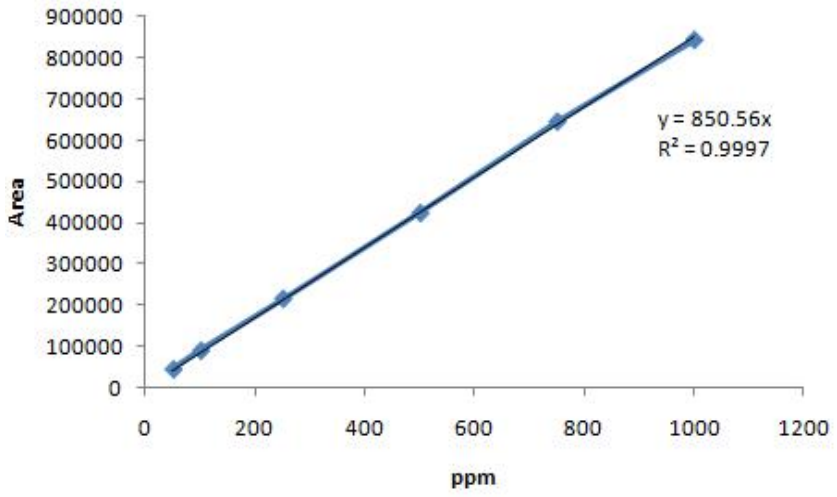


Figure F.2 Acetic acid standard calibration curve (concentrations measured by Shimadzu 10A series high performance liquid chromatography).

APPENDIX G

GLUTAMIC ACID ANALYSIS

G1. PITC (Phenyl Isothiocyanate) Derivatization Reaction

1. 100 µl of 100 mmol/l phenyl isothiocyanate (acetonitrile solution) and 100 µl of 1 mol/l triethylamine (acetonitrile solution) were added to 200 µL of the liquid sample. The mixture is stirred and let to completely react for 20 minutes at 40^0C.
2. Cool the mixture to room temperature after incubation. Add aqueous hydrochloric acid solution to neutralize the solution (adjust pH to 7.0).
3. Finally, extract the derivatized sample with n-Hexane (3:4).
4. Separate the aqueous phase for injection to the HPLC.

G2. HPLC operation conditions

Mobile Phases

MP-A: 10 mmol/l (sodium) phosphate buffer (pH 7.0)

MP-B: Acetonitrile

Flow Rate: 1.0 ml/min

Gradient Profile:

Table G.1. HPLC gradient pump operation profile.

Time	Module	Action	Concentration
5.00	Pumps	B. Concentration	10
7.50	Pumps	B. Concentration	25
7.51	Pumps	B. Concentration	100
13.00	Pumps	B. Concentration	100
13.01	Pumps	B. Concentration	5
20.00	Controller	Stop	

Column Temperature: 40^0C

Detection: Absorbance at 254 nm

Injection Volume: 10 µl

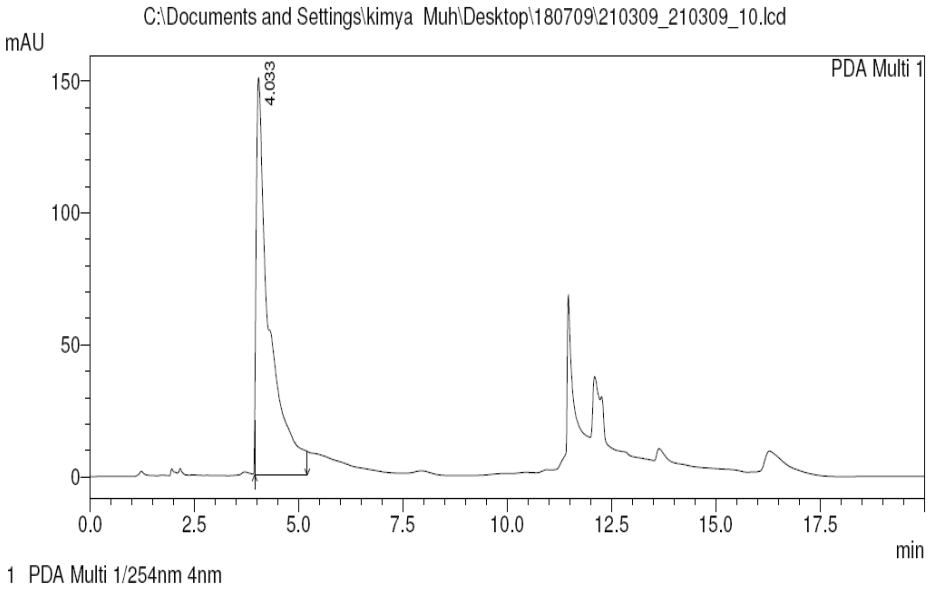
Column: Teknokroma mediterranea Sea 18 (150 mm X 4.6 mm i.d, 3µm)

G3. PITC Derivatization and HPLC Analysis Chemical Compositions

Table G.2. Glutamic acid measurement mobile phase and derivatization chemicals composition.

	Composition	Concentration	Volume	Amount	pH
Mobile phase A	KH_2PO_4 and K_2HPO_4	10 mM	1 l	KH_2PO_4 (1.169 g) K_2HPO_4 (2.438 g)	7.0
Mobile phase B	Pumps	N/A	1 l		N/A
TEA	Pumps	1.0 M	100 ml	13.9 ml	N/A
PITC	Pumps	100 mM	100 ml	1.2 ml	N/A

G4. Sample Glutamic Acid Measurement HPLC Chromatogram



Quantitative Results

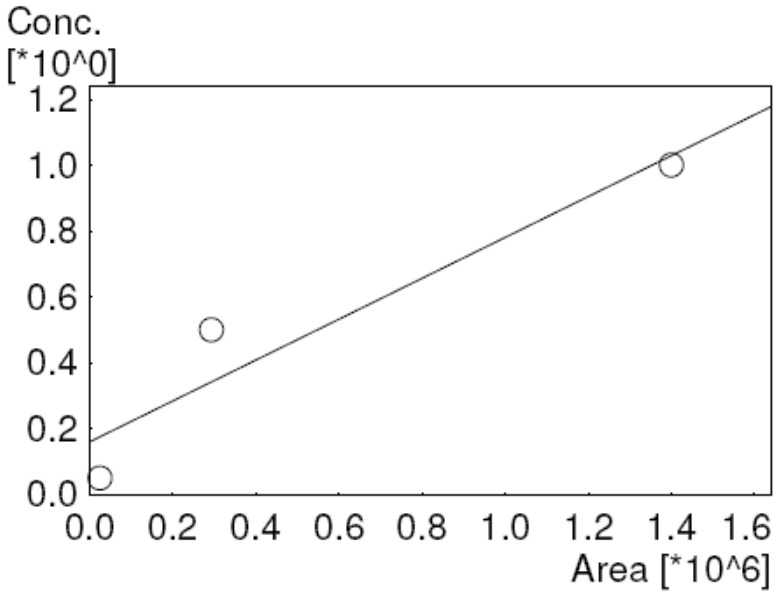
PDA

ID#	Name	Ret. Time	Area	Height
1	RT5.164	4.033	3252261	150626

Conc.
2.181

Figure G.1 Glutamic acid standard calibration curve (concentrations measured by Shimadzu 20A series high performance liquid chromatography).

G5. Sample Glutamic Acid HPLC Calibration Curve



#	Conc (Ratio)	Area	Area
1	0.050	25122.1	25122
2	0.500	292947.6	292948
3	1.000	1400269.3	1400269

Figure G.2 Glutamic acid standards calibration curve (concentrations measured by Shimadzu 20A series high performance liquid chromatography).

APPENDIX H

DETERMINATION OF BACTERIOCHLOROPHYLL *a* CONTENT

This section describes the calculation procedure to determine bacteriochlorophyll.

Bacteriochrophyll *a* properties

molecular formula = $C_{55}H_{74}N_4O_6Mg$

molecular weight (MW) = 911.5 g/mol

Extinction coefficient =76 $mM^{-1}cm^{-1}$

Beer Lambert's Law: $A = \varepsilon C L$

where A: absorbance

ε : Extinction coefficient ($mM^{-1}cm^{-1}$)

C: concentration (mM)

L: length(cm)

Thus, $C = \frac{A}{\varepsilon \times L}$

Shown below is a sample calculation for a 0.782 absorbance spectrophotometric reading at 770 nm.

$$\text{bchl } a \text{ content} = \left(\frac{0.782}{76 mM^{-1} cm^{-1} \times 1 cm} \right) \times \left(911.5 \frac{mg}{mmol} \right) = 9.39 \frac{mg}{l_{bchla}}$$

APPENDIX I

SAMPLE CALCULATIONS FOR HYDROGEN PRODUCTION

In this section, the calculation procedure for hydrogen productivity, substrate conversion efficiency (yield) and light conversion efficiency is shown. To illustrate, the Phase II data for the continuous panel photobioreactor with 40 mM/2 mM Ac/Glu feed given in section 4.3.1 will be used. The culture volume is 7.6 l and 84.4 l of hydrogen gas was produced. 50.6 g of acetate and 6.5 g of glutamate were utilized over the 31 days. The 0.4 m^2 reactor surface was continuously illuminated by tungsten lamps providing 86 W/m^2 light intensity. These data are used to determine the hydrogen productivity, substrate conversion and light conversion efficiency.

I1. Hydrogen Productivity

This is the rate at which hydrogen gas is produced by the bacterial culture. It can be expressed in terms of the volume or moles of hydrogen gas produced.

I1.1 Volumetric Productivity

The volumetric hydrogen productivity is the rate at which hydrogen is produced in terms of volume. It is expressed as ml $H_2/l_c.h$.

$$\text{Volumetric Hydrogen Productivity} = \left(\frac{\text{Volume of hydrogen produced (ml)}}{\text{Volume of culture} \times \text{Time}} \right) \quad \text{(I1.1)}$$

$$\text{Volume of hydrogen produced} = \left(V_{final} - V_{initial}\right) \times y_{H_2} \qquad \text{(I.1.2)}$$

where V is the volume (ml) of the total gas in the collection column and y_{H_2} is the mole fraction of hydrogen in the total gas produced.

A sample calculation for the volumetric productivity using the Phase II data for the 40 mM/ 2 mM Ac/Glu fed CPBR is shown.

$$\text{Volumetric Hydrogen Productivity} = \left(\frac{84.4 \text{ l } H_2 \times 1000 \frac{\text{ml } H_2}{\text{l } H_2}}{7.6 \text{ l}_c \times 31 \text{ days} \times 24 \frac{\text{h}}{\text{day}}} \right) = 15 \text{ ml } H_2 / \text{l}_c\text{.h}$$

I1.2 Molar Productivity

This is the rate at which hydrogen gas is produced in terms of moles. It is expressed as mmol H_2/l_c.h. It determining the molar productivity, several assumptions are made. They are:

1. The total gas collected obeys the ideal gas law.
2. There is no leakage from or into the gas collection column and the photobioreactor.
3. The atmospheric pressure is constant at 687 mmHg.

Using the fist assumption, the moles of hydrogen produced can be determined using the ideal gas equation.

$$PV = NRT \qquad \text{(I1.3)}$$

Equation H1.3 can be rearranged as:

$$N = CV \qquad \text{(I1.4)}$$

where $C = \frac{P}{RT}$ is a conversion factor (concentration)

The conversion factor C was determined at the temperature range experienced in the outdoor conditions. The values obtained considering Ankara's atmospheric pressure 687 mmHg (Berktaş and Bircan, 2003) and at 760 mmHg are shown in Table I1.

Table I.1: Molar productivities conversion factor.

T (^oC)	**C(mmol/ml) 687 mmHg**	**C(mmol/ml) 760 mmHg**
5	0.040	0.044
10	0.039	0.043
15	0.038	0.042
20	0.038	0.042
25	0.037	0.041
30	0.037	0.040
35	0.036	0.040
40	0.035	0.039
45	0.035	0.038

It is observed that the conversion factor remains more or less the same at 0.04 mmol/ml. The molar productivities were determined using the conversion factor (C= P/RT) 0.0445 mmol/ml. Koku (2001) used 0.037 mmol/ml while Uyar (2008) used 0.0446 mmol/ml.

$$\text{Molar Productivity (N)} = \text{Conversion Factor} \times \text{Volumetric Productivity} \quad \text{(I1.5)}$$

A sample calculation for the molar productivity using the Phase II data for the 40 mM/ 2 mM Ac/Glu fed CPBR is shown.

$$\text{Molar Productivity} = 0.0445 \ \frac{\text{mmol}}{\text{ml}} \times 15 \text{ ml } H_2/l_c.h$$
$$= 0.7 \text{ mmol } H_2/l_c.h$$

I2. Substrate Conversion Efficiency (Yield)

The substrate conversion efficiency (yield) determines how effectively the substrate (organic acid) fed to the bacterial culture is used in hydrogen production. It is the ratio of the amount hydrogen produced to the amount of theoretical hydrogen produced from the given carbon sources. Acetate and glutamate were the two carbon sources used. Shown in Equation (I2) is the substrate conversion (yield) determination.

$$\text{Substrate conversion efficiency} = \frac{\text{Actual moles of } H_2 \text{ produced}}{\text{Theorectical moles of } H_2 \text{ produced}} \times 100 \quad \text{(I2)}$$

$$= \frac{\text{moles of } H_2 \text{ produced}}{4 \times \text{moles of Acetate Utilized} + 10 \times \text{moles of Acetate Utilized}}$$

A sample substrate conversion efficiency (yield) calculation for the 40 mM/ 2 mM Ac/Glu fed CPBR Phase II data is shown.

$$\text{Yield} = \frac{84.4\ \text{l} \times 0.089 \frac{\text{g}}{\text{l}} \times \frac{1}{2\ \text{g/mol}}}{(4 \times \frac{50.6\ \text{g}}{60.05\ \text{g/mol}}) + (10 \times \frac{6.5\ \text{g}}{147.13\ \text{g/mol}})} \times 100 = 99\%$$

I3. Acetate Conversion

$$\text{Acetate Conversion} = \left(\frac{\text{Acetate Input (mM) - Acetate Output (mM)}}{\text{Acetate Input (mM)}} \times 100 \right) \quad \text{(I3)}$$

In the Phase II data for the 40 mM/2 mM Ac/Glu fed CPBR described at the beginning of the Appendix I, daily 40 mM acetate was fed and an average of 2 mM acetate was removed as shown in the organic acid analysis Figure 4.21.

$$\text{Acetate Conversion} = \frac{40\text{ mM - }2\text{ mM}}{40\text{ mM}} \times 100 = 95\%$$

I4. Light Conversion Efficiency

$$\text{Light Conversion Efficiency } (\eta) = \frac{33.61 \times \rho_{H_2} \times V_{H_2}}{I \times A \times t} \times 100 \qquad \text{(I4)}$$

where ρ_{H2} is the density of the produced hydrogen gas in g/l, V_{H2} is the volume of produced hydrogen gas in l, I is the light intensity in W/m^2, A is the irradiated area in m^2 and t is the duration of hydrogen production in hours.

Using the Phase II data for the continuous panel photobioreactor with 40 mM/2 mM Ac/Glu feed.

$$\text{Light Conversion Efficiency } (\eta) = \frac{33.61 \times 0.089 \frac{g}{l} \times 84.4\text{ l}}{86 \frac{W}{m^2} \times 0.4 m^2 \times 31\text{ days} \times 24 \frac{h}{day}} \times 100 = 0.99\%$$

APPENDIX J

CHEMICAL COMPOSITION AND SEM OF THE ORIGINAL GÖRDES CLINOPTILOLITE

Table J.1 Chemical composition of the original clinoptilolite sample.

Component	% Weight	Moles/100 g	Exchangeable Cations meq/g
SiO_2	68.2	1.137	
Al_2O_3	11.2	0.110	
Fe_20_3	2.400	0.150	
MgO	1.000	0.025	0.500
CaO	2.100	0.037	0.740
Na_2O	0.600	0.010	0.200
K_2O	4.400	0.046	0.920
MnO	0.100	0.001	
TiO_2	0.100	0.001	
P_2O_5	<0.100	<0.001	
Total	90.100		2.36

(Aşiroğlu, 2006)

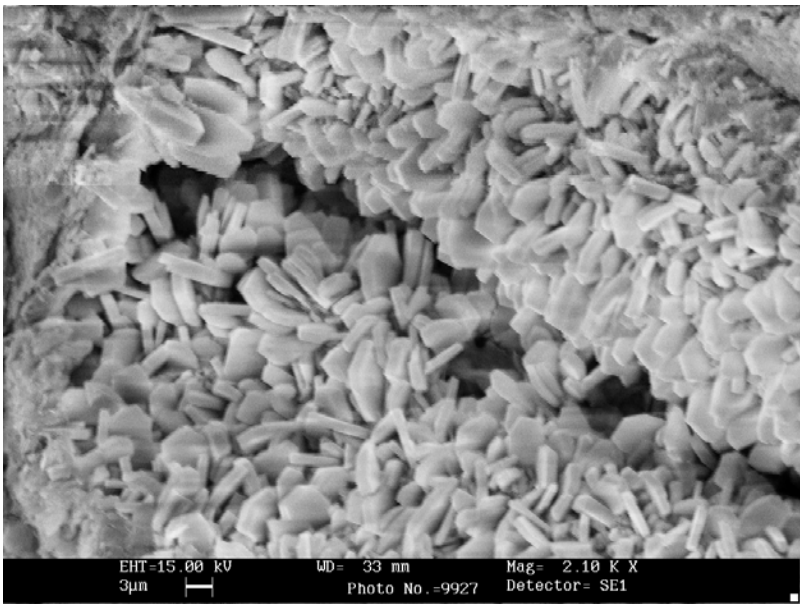


Figure J.2 Scanning Electron Micrograph (SEM) of the original Gördes clinoptilolite (Aşiroğlu, 2006).

APPENDIX K

DARK FERMENTER EFFLUENT OF SUGAR-BEET THICK JUICE MOLASSES

K1. Composition of the DFE of Sugar-Beet Thick Juice Molasses

Table K.1 The composition of the DFE of sugar-beet thick juice molasses.

Composition	Amount (mM)
Acetate	95
Lactate	128
Sucrose	2
NH_4Cl	12.4

K2. Ammonium Ion Electrode Calibration Curve

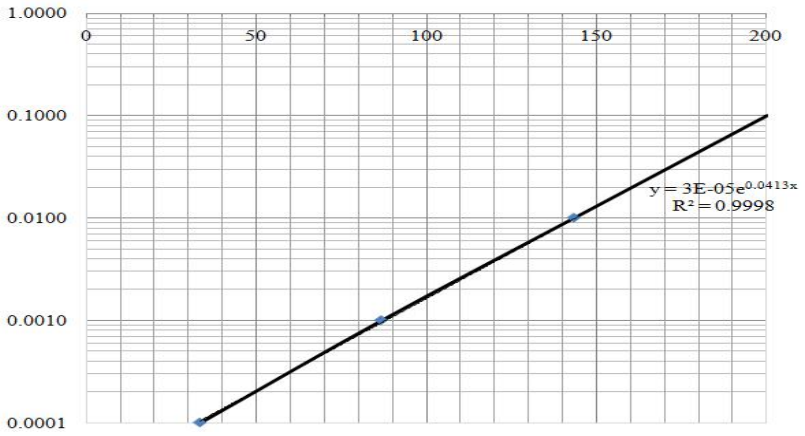


Figure K.1 Ammonium ion electrode (Russel Model 95-5129) calibration curve.

APPENDIX L

PRELIMINARY STUDY ON THE USE OF PHOTOBIOREACTOR EFFLUENT AS FERTILIZER

A test on the use of photobioreactor effluent as a fertilizer was carried out. The effluent was taken from an 80 l tubular photobioreactor (Boran *et al*., 2009) containing *R. capsulatus* DSM 1710 wild type. Suumer wheat seeds were used in the experiment.

Two pots, one watered using the effluent from the photobioreactor and the other using tap water were run parallel to each other in indoor conditions. The pots that contained 10 wheat seeds each were run in duplicates and put next to a window to receive sunlight. Regularly, they were placed outside under direct sunlight but were returned in indoor conditions.

At the start of the experiment, the PBR effluent had an O.D. of 0.440 and pH of 7.782. The pots were watered with 100 ml of the PBR effluent and tap water after every two days.

Shown in Figures L.1 and L.2 are pictures of the pots with the wheat seeds and a comparison of the pots with respect to the shoot length of the wheat plant. Germination of the seeds was observed on the 2^{nd} day after the start of the experiment. The wheat seedlings shoots were observed on the 6^{th} day. Quicker growth and longer shoots were observed in the pots watered with the PBR effluent. However, rust formation was observed after the 41^{st} day in the pots with the the PBR effluent. This fungal infection spread to the other pots. Due to the fungal infection, the leaflets shriveled as shown in Figure L.1 (d). The tap watered wheat plants showed better resilience to the fungal infections as the length of the shoots surpassed that of the PBR effluent watered pots as seen in Figure L.2.

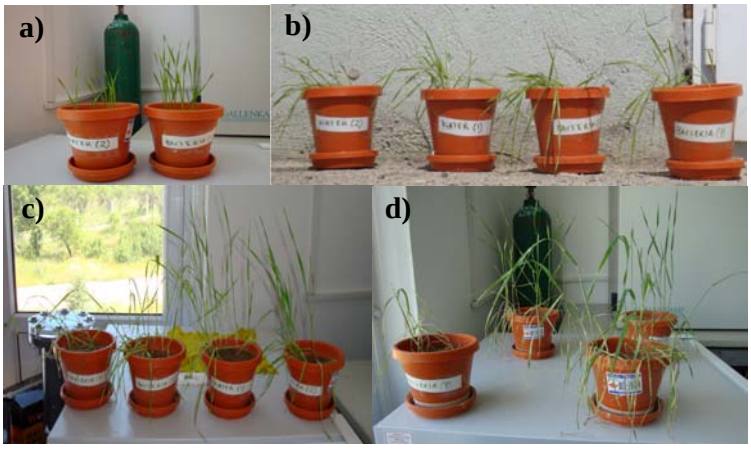


Figure L.1 a) Comparison of the pots after wheat germination (water, PBR effluent from left to right). b) The pots placed in outdoor (water (1) and (2), PBR effluent (1) and (2) from left to right). c) The pots placed in indoor conditions (PBR effluent (1) and (2), water (1) and (2) from left to right). d) The wheat plant after fungus infection (PBR effluent (1) and (2) in front and water (1) and (2) at the back).

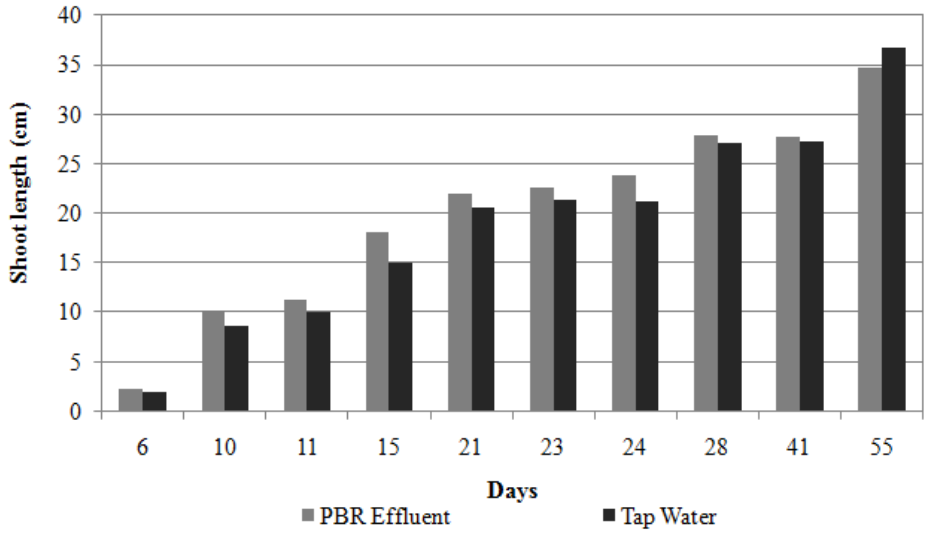


Figure L.2 Comparison of the wheat plant shoot lengths grown using the PBR effluent and tap water.

APPENDIX M

NATURAL ADAPTATION OF *R. capsulatus* in OUTDOOR CONDITIONS

Successful operation of photobioreactors for hydrogen production in outdoor conditions under natural sunlight and varying day and night cycle involve the use of PNS bacteria that is able to withstand the varying outdoor temperatures and light intensity. The PNS bacteria *Rhodobacter capsulatus* (DSM 1710) was adapted to real life conditions. The experiment, carried out by the METU Biohydrogen group members, Muazzez Gürgan, Begüm Peksel and Nilüfer Afşar, aimed to adapt *Rhodobacter capsulatus* (DSM 1710) to the high summer temperatures experienced during summer in Ankara. Described below is the experimental procedure carried out between July-August 2008 in Ankara, Turkey.

- 10% *Rhodobacter capsulatus* (DSM 1710) wildtype was innoculated into 50 ml bottles containing 15 mM/10 mM Ac/Glu growth medium under sterile conditions.
- Two duplicate bottles were placed outside, under direct sunlight and in a glass house.
- Daily measurements done included:
  - Temperature measurement of the outdoor, culture and the greenhouse.
  - pH and OD at 660 nm.
- When OD reached 1.0, the bacteria was passage into fresh media.

Successful adaptation with bacterial growth and hydrogen production observed at 41^0C was achieved after 6 passages.

APPENDIX N

QUANTITATIVE ANALYSIS OF PHB USING GAS CHROMATOGRAPHY

Poly-3-hydroxybutyrate is a biodegradable thermoplastic formed as an intracellular and carbon storage material by many genera of bacteria. It is formed as crystalline granules at amounts of 65% or more of the dry weight of the cells (Senior and Dawes, 1973).

In this study, PHB formation by *Rhodobacter capsulatus* was measured by gas chromatography. Quantitative analysis of PHB using GC is based on the conversion of PHB to methyl ester by acidic methanolysis reaction and examination of the volatile methyl ester by GC. Hydrolysis and esterification of PHB to its methyl ester form is done by treatment with chlorofoam, methanol and sulfuric acid mixture at high temperature. The amount of PHB is recovered in the chlorofoam phase (organic phase) after methanolysis by addition of water.

The analysis method used in this study was developed by Braunegg *et al*., 1978. It involves methanolysis of the PHB containing biomass and GC measurement of the methyl hydroxybutyric acid (ME-3HB). In an optimization procedure done by Selma Bulut, the PHB concentration of biomass sample taken from the 60 mM/ 4 mM Ac/Glu fed media CPBR was measured. 150 ml and 200ml biomass sample with O.D. of 1 were placed in 250 ml vials and centrifuged (Sigma- model 4K15) at 10000g for 20 minutes at 4^0C. The packed cell 0.7 g and 1.2 g wet weight for the 150 ml and 200 ml samples respectively, were used in the analysis. Also, 4 ml of 2, 5, 10 and 15 mg PHB standards (Sigma) samples were measured to construct a standard calibration curve.

In the procedure, the first step was methanolysis. The PHB standards and centrifuged CPBR samples were placed in rubber-stopper bottles and 2 ml $MeOH/H_2SO_4$ (3%/10% v/v) and 2 ml Chlorofoam solutions added. The

rubber-stopper bottles were covered with aluminium foils to prevent exposing the chlorofoam solution to light. The bottles were then dried in an oven at 100^0C for 3.5 h. During drying, at intervals of 1 hour, the samples were shaken for 1-2 minutes to homogenize the mixture. After methanolysis, the bottles were cooled to room temperature and the water layer was removed leaving behind a solution containing methanol, chlorofoam and ME-3HB produced during methanolysis. ME-3HB is soluble in chlorofoam but insoluble in water.

In the second step, the ME-3HB was measured using GC (Agilent 6890 GC) equipped with an FAB capillary column connected to an FID system. The injector and detector temperatures were set at 230^0C and 250^0C respectively. The oven temperature was set at 70^0C for 1 minute and then increased at a rate of 8^0C per minute until 160^0C. 1µl sample of the methanolysed samples were injected in split mode (1/20). Shown in Figure N.1 is a smplae chromatogram for the 10 mg sample PHB standard. Methanol, chlorofoam and ME-3HB peaks are seen at the 3.1, 3.7 and 9.7 minutes respectively.

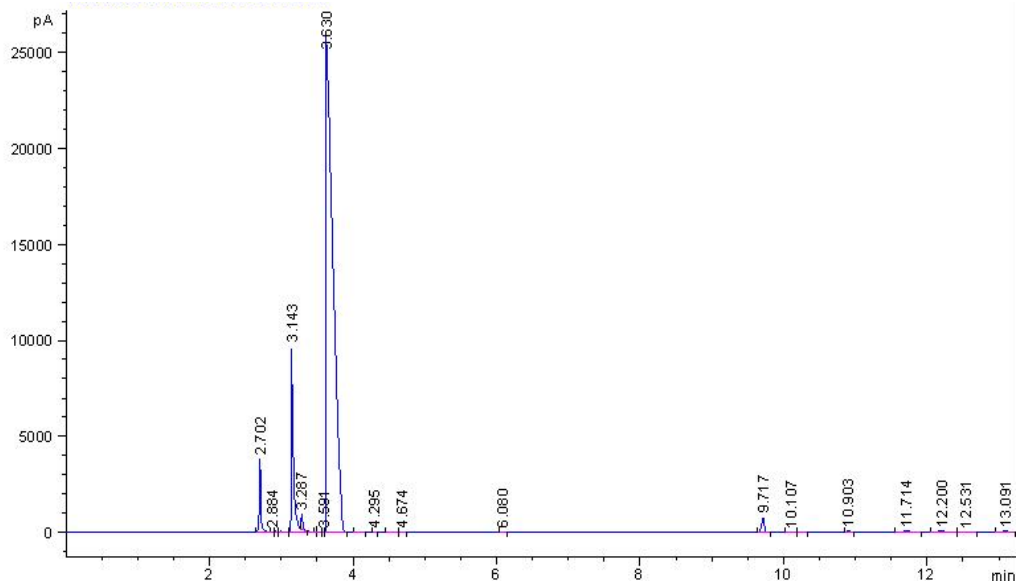


Figure N.1 Gas chromatogram for the 10 mg PHB standard.

Shown in Table N.1 and Figure N.2 are the results of the PHB standards measurement using gas chromatography and the calibration curve.

Table N.1 Results of the PHB standards measurement using gas chromatography.

Standard (mg)	PHB content (mg/ml) after GC analysis	Peak Area
2	500	304.49
5	1250	787.21
10	2500	1990.30
15	3750	2695.00

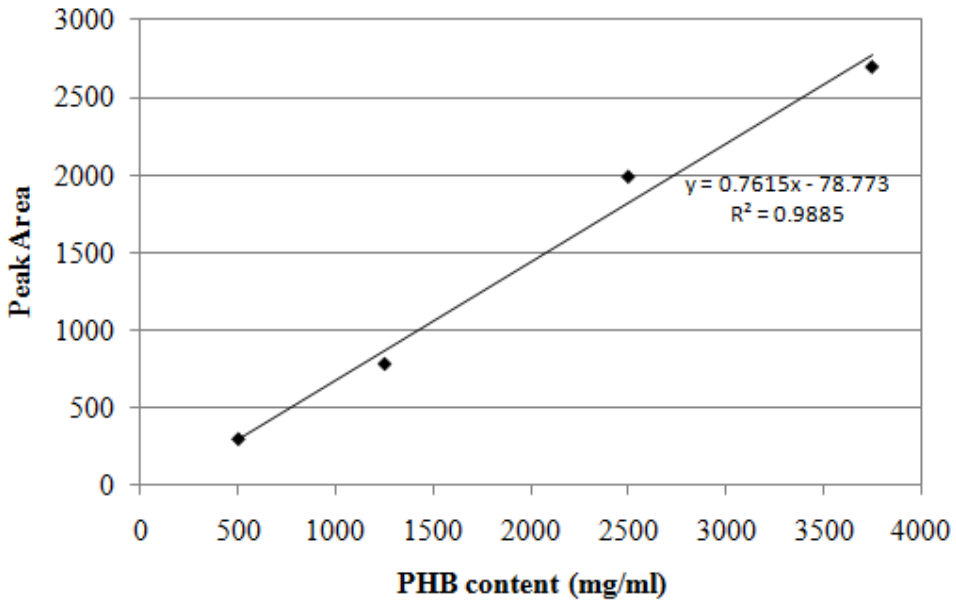


Figure N.2 Gas chromatography PHB measurement standards calibration curve.

The determination of the PHB content is illustrated using the 2 mg standard (4 ml methanolysed) sample.

$$\text{PHBcontent} = \frac{2 \times 10^3 \text{mg}}{4 \text{ ml}} = 500 \frac{\text{mg}}{\text{ml}} \quad \text{(N1)}$$

Shown in Table N.2 are the gas chromatography PHB measurement results of the of the samples taken from the 60 mM/ 4 mM Ac/Glu fed media CPBR.

Table N.2 Gas chromatography PHB measurement results of the 60 mM/ 4 mM Ac/Glu fed media CPBR.

Volume (ml)	**Wet weight (mg)**	**PHB concentration after GC analysis(mg/ml)**	**PHB content (%w/w)**
150	700	0.632	0.36
200	1200	0.736	2.44

A sample calculation of the PHB content is illustrated using the 150 ml sample (4 ml methanolysed) sample from the 60 mM/ 4 mM Ac/Glu fed media CPBR.

$$\text{PHB Content} = \frac{\text{Mass of PHB}}{\text{Wet weight of sample}} \quad \text{(N2)}$$

$$= \frac{0.632 \text{ mg } \frac{\text{PHB}}{\text{ml}} \times 4\text{ml}}{700 \text{ mg}} \times 100 = 0.36\%$$

It was observed that the amount of PHB obtained from the CPBR sample was very low. This could be attributed to the low amounts recovered (extracted) after methanolysis. It is suggested that lyophilization before methanolysis would enable the recovery of higher amounts of PHB. The presence of water reduces the efficiency of methanolysis reaction. It is shown that the addition of Na_2SO_4 after extraction increased efficiency and peak area (Suludere, 2001).

APPENDIX O

EXPERIMENTAL DATA

Table O.1 OD, cell dry weight and pH values for *R. capsulatus* (DSM 1710) outdoor CPBR.

Day	Date	OD	gdw/l_c	pH	Cumulative Total Gas Produced (l)
1	13/09/08	0.782	0.424	6.856	0.00
2	14/09/08	1.520	0.825	7.494	0.46
3	15/09/08	1.830	0.994	7.55	3.58
4	16/09/08	1.880	1.021	7.62	5.41
5	17/09/08	1.668	0.905	7.393	6.33
6	18/09/08	1.960	1.064	7.685	6.64
7	19/09/08	1.766	0.958	7.720	7.32
8	20/09/08	1.896	1.029	7.622	7.55
9	21/09/08	2.075	1.126	7.620	8.47
10	22/09/08	1.830	0.993	7.637	10.03
11	23/09/08	1.808	0.981	7.617	11.22
12	24/09/08	1.798	0.976	7.590	12.32
14	26/09/08	1.938	1.052	7.381	14.52
15	27/09/08	1.844	1.001	7.365	15.72
16	28/09/08	1.803	0.978	7.327	16.36
17	29/09/08	1.818	0.987	7.335	16.82
18	30/09/08	1.768	0.959	7.312	17.18
19	01/10/08	1.848	1.003	7.399	18.01
20	02/10/08	2.080	1.129	7.464	18.28
21	03/10/08	1.772	0.962	7.485	18.65

Table O.2 HPLC data for the *R. capsulatus* (DSM 1710) outdoor CPBR.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid	
		mM	Area	mM	Area	mM	mM	Area	mM
1	13/09/08	1667	0.021	-	-	1041715	20.916	-	-
2	14/09/08	-	-	-	-	784000	14.260	3666	0.071
3	15/09/08	-	-	-	-	447488	7.848	-	-
5	17/09/08	1267	0.016	2141	0.037	267903	5.379	5346	0.103
7	19/09/08	4616	0.057	18306	0.318	158885	3.190	-	-
9	21/09/08	2996	0.037	1687	0.029	134764	2.706	-	-
10	22/09/08	5497	0.068	3326	0.058	137101	2.753	-	-
11	23/09/08	-	-	-	-	172494	3.463	4282	0.083
12	24/09/08	-	-	2889	0.050	208539	4.187	5188	0.100
14	26/09/08	1599	0.020	8386	0.146	153186	3.076	12302	0.237
15	27/09/08	-	-	5071	0.088	220411	4.425	13118	0.253
16	28/09/08	2808	0.035	4592	0.080	262036	5.261	12692	0.245
17	29/09/08	-	-	2371	0.041	332406	6.674	15825	0.305
18	30/09/08	-	-	1867	0.032	397622	7.984	17521	0.338
19	01/10/08	-	-	-		418037	8.393	15997	0.308
20	02/10/08	3157	0.039	6537	0.113	393543	7.902	15229	0.293
21	03/10/08	1609	0.020	4513	0.078	483635	9.710	14510	0.280

Table O.3 OD, cell dry weight and pH values for the heat adapted *R. capsulatus* (DSM 1710) outdoor CPBR.

Day	Date	OD	Biomass (gdcw/l_c)	pH	Cumulative Total Gas Produced (l)
1	20/09/08	0.392	0.213	6.771	0.00
2	21/09/08	0.524	0.284	6.917	0.00
3	22/09/08	0.824	0.447	7.164	0.00
4	23/09/08	0.888	0.482	7.213	0.28
5	24/09/08	1.248	0.677	7.411	0.28
7	26/09/08	1.730	0.939	7.445	0.28
8	27/09/08	1.634	0.887	7.343	3.21
9	28/09/08	1.622	0.880	7.312	4.29
10	29/09/08	1.692	0.918	7.294	5.45
11	30/09/08	1.660	0.901	7.259	6.48
12	01/10/08	1.710	0.928	7.410	6.81
13	02/10/08	1.812	0.983	7.596	7.39
14	03/10/08	1.750	0.950	7.754	7.96
15	04/10/08	2.105	1.142	7.772	8.38
16	05/10/08	1.936	1.051	7.780	8.79
17	06/10/08	1.866	1.013	7.723	9.61
18	07/10/08	1.856	1.007	7.782	10.60
19	08/10/08	1.790	0.971	7.692	11.18
21	10/10/08	1.882	1.021	7.635	11.84
22	11/10/08	2.060	1.118	7.598	13.24
25	14/10/08	1.958	1.063	7.629	13.66
27	16/10/08	1.806	0.980	7.506	15.55
28	17/10/08	1.864	1.012	7.430	16.21
30	19/10/08	1.776	0.964	7.468	16.79
31	20/10/08	1.676	0.910	7.363	17.70
32	21/10/08	1.642	0.891	7.347	18.53
34	23/10/08	1.796	0.975	7.367	19.52
35	24/10/08	1.648	0.894	7.299	21.66
36	25/10/08	1.618	0.878	7.259	22.16
37	26/10/08	1.508	0.818	7.271	23.06

Table O.3 (Continued).

Day	Date	OD	Biomass ($gdcw/l_c$)	pH	Cumulative Total Gas Produced (l)
38	27/10/08	1.498	0.813	7.254	23.64
39	28/10/08	1.470	0.798	7.212	23.64
40	29/10/08	1.378	0.748	7.237	23.72
41	30/10/08	1.520	0.825	7.314	23.97
42	31/10/08	1.708	0.927	7.364	24.30
43	01/11/08	1.740	0.944	7.396	24.88
44	02/11/08	1.624	0.881	7.381	25.21
45	03/11/08	1.572	0.853	7.412	25.62
46	04/11/08	1.530	0.830	7.417	26.03
48	06/11/08	1.462	0.793	7.327	26.45
49	07/11/08	1.380	0.749	7.275	26.45
50	08/11/08	1.661	0.901	7.281	26.45
52	10/11/08	1.768	0.959	7.280	26.45
53	11/11/08	1.642	0.891	7.293	26.86
54	12/11/08	1.410	0.765	7.173	27.44
55	13/11/08	1.278	0.694	7.165	27.93
56	14/11/08	1.234	0.670	7.459	28.10
57	15/11/08	1.268	0.688	7.178	28.34
59	17/11/08	1.332	0.723	7.209	28.84
60	18/11/08	1.240	0.673	7.194	29.01
61	19/11/08	1.302	0.707	7.161	29.17
62	20/11/08	1.292	0.701	7.251	29.34
63	21/11/08	1.264	0.686	7.251	29.75
64	22/11/08	1.210	0.657	7.239	30.33
66	24/11/08	1.268	0.688	7.251	30.66
67	25/11/08	1.420	0.771	7.359	31.32
68	26/11/08	1.588	0.862	7.334	32.14
69	27/11/08	1.412	0.766	7.390	32.55
70	28/11/08	1.322	0.717	7.335	32.80
71	29/11/08	1.423	0.772	7.436	33.38
73	01/12/08	1.360	0.738	7.457	34.62
74	02/12/08	1.356	0.736	7.384	35.11
76	04/12/08	1.482	0.804	7.403	36.10
77	05/12/08	1.444	0.784	7.345	36.60
79	07/12/08	1.366	0.741	7.418	37.42

Table O.3 (Continued).

Day	Date	OD	Biomass (gdcw/l_c)	pH	Cumulative Total Gas Produced (l)
80	08/12/08	1.438	0.780	7.382	37.42
81	09/12/08	1.654	0.898	7.402	37.67
82	10/12/08	1.488	0.808	7.477	37.92
83	11/12/08	1.516	0.823	7.392	38.25
86	14/12/08	1.444	0.784	7.446	39.24
87	15/12/08	1.426	0.774	7.445	39.57
88	16/12/08	1.294	0.702	7.520	39.73
89	17/12/08	1.234	0.670	7.296	39.81
90	18/12/08	1.157	0.628	7.249	39.81
92	20/12/08	0.937	0.509	7.360	40.81
94	22/12/08	1.070	0.581	7.296	40.97
96	24/12/08	0.948	0.514	7.323	41.05
101	29/12/08	0.928	0.504	7.370	41.71

Table O.4 HPLC data for the heat adapted *R. capsulatus* (DSM 1710) outdoor CPBR.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	Area	mM
1	20/09/08	2540	0.039	-	-	1008859	20.667	-	-	-	-
1	20/09/08	-	-	-	-	962719	19.722	-	-	-	-
2	21/09/08	2613	0.040	-	-	916007	18.765	2033	0.022	-	-
4	23/09/08	-	-	-	-	542975	11.123	-	-	-	-
7	26/09/08	1399	0.022	10693	0.159	286081	5.861	-	-	6078	0.115
8	27/09/08	1114	0.017	8871	0.132	414999	8.502	-	-	8834	0.167
9	28/09/08	1655	0.025	8172	0.122	491581	10.070	-	-	10984	0.208
10	29/09/08	3305	0.051	5211	0.078	343487	7.037	-	-	9339	0.177
11	30/09/08	2216	0.034	2950	0.044	370556	7.591	-	-	8260	0.157
12	01/10/08	1179	0.018	1031	0.015	473848	9.707	-	-	9348	0.177
13	02/10/08	7334	0.113	3571	0.053	508905	10.425	-	-	8770	0.166
14	03/10/08	6367	0.098	2598	0.039	322887	6.615	-	-	4672	0.089
15	04/10/08	6113	0.094	4405	0.066	284383	5.826	-	-	3041	0.058
16	05/10/08	8979	0.138	5448	0.081	282081	5.779	-	-	3144	0.060
17	06/10/08	6284	0.097	2075	0.031	285178	5.842	-	-	2440	0.046
18	07/10/08	7547	0.116	2881	0.043	378755	7.759	-	-	2402	0.046
21	10/10/08	2620	0.040	4510	0.067	270993	5.551	10284	0.114	3185	0.060
22	11/10/08	2138	0.033	4013	0.060	304586	6.240	9141	0.101	5689	0.108
25	14/10/08	2365	0.036	6192	0.092	225390	4.617	12572	0.139	20020	0.379
27	16/10/08	2003	0.031	4592	0.068	186607	3.823	7884	0.087	18801	0.356
28	17/10/08	1672	0.026	4093	0.061	259870	5.324	9941	0.110	19691	0.373

Table O.4 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	Area	mM
30	19/10/08	1929	0.030	6690	0.100	328216	6.724	15390	0.170	29226	0.554
32	21/10/08	1260	0.019	8497	0.127	404173	8.280	10212	0.113	27840	0.528
34	23/10/08	2202	0.034	6965	0.104	407749	8.353	10944	0.121	32880	0.623
35	24/10/08	2935	0.045	7671	0.114	481664	9.867	8860	0.098	30070	0.570
36	25/10/08	2083	0.032	1577	0.024	610280	12.502	10867	0.120	28308	0.536
37	26/10/08	1849	0.028	6258	0.093	665865	13.641	-	-	30111	0.571
38	27/10/08	1364	0.021	5125	0.076	722342	14.798	-	-	29628	0.561
39	28/10/08	1530	0.024	4744	0.071	786343	16.109	-	-	28860	0.547
40	29/10/08	3280	0.050	5124	0.076	799993	16.388	1031	0.011	29626	0.561
41	30/10/08	10450	0.161	1032	0.015	725027	14.853	-	-	30246	0.573
43	01/11/08	1693	0.026	4434	0.066	644114	13.195	-	-	32610	0.618
44	02/11/08	1854	0.029	8412	0.125	571399	11.705	-	-	37391	0.708
45	03/11/08	1589	0.024	13097	0.195	482367	9.882	-	-	41447	0.785
46	04/11/08	2122	0.033	3681	0.055	450792	9.235	1334	0.015	69108	1.309
48	06/11/08	1557	0.024	17058	0.254	390823	8.006	-	-	52533	0.995
49	07/11/08	-	-	25843	0.385	293211	6.007	-	-	51003	0.966
50	08/11/08	1753	0.027	27717	0.413	236430	4.843	-	-	45290	0.858
52	10/11/08	1032	0.016	19109	0.285	200251	4.102	-	-	41422	0.785
53	11/11/08	1499	0.023	7628	0.114	109653	2.246	-	-	35608	0.675
54	12/11/08	1360	0.021	4750	0.071	64860	1.329	-	-	26047	0.494
55	13/11/08	1124	0.017	1679	0.025	35888	0.735	-	-	19736	0.374

Table O.4 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	Area	mM
56	14/11/08	-	-	-	-	11385	0.233	-	-	9145	0.173
57	15/11/08	-	-	-	-	3684	0.075	-	-	5255	0.100
59	17/11/08	-	-	-	-	41605	0.852	-	-	1976	0.037
60	18/11/08	-	-	-	-	93837	1.922	-	-	3376	0.064
61	19/11/08	-	-	-	-	218427	4.475	-	-	5380	0.102
62	20/11/08	-	-	-	-	385545	7.898	-	-	7808	0.148
63	21/11/08	-	-	-	-	388076	7.950	-	-	11625	0.220
64	22/11/08	-	-	-	-	459710	9.417	-	-	16630	0.315
66	24/11/08	-	-	-	-	483812	9.911	-	-	19376	0.367
67	25/11/08	-	-	3959	0.059	520181	10.656	1344	0.015	31237	0.592
68	26/11/08	-	-	5389	0.080	502507	10.294	-	-	31856	0.604
69	27/11/08	-	-	3824	0.057	521268	10.679	-	-	31363	0.594
70	28/11/08	-	-	-	-	530697	10.872	1344	0.015	30351	0.575
73	01/12/08	-	-	-	-	576767	11.815	1229	0.014	28576	0.541
74	02/12/08	-	-	9386	0.140	560834	11.489	1498	0.017	38934	0.738
76	04/12/08	-	-	9616	0.143	588084	12.047	1554	0.017	35181	0.667
77	05/12/08	-	-	9467	0.141	563511	11.544	2242	0.025	42589	0.807
79	07/12/08	-	-	9589	0.143	607224	12.439	3078	0.034	60052	1.138
80	08/12/08	-	-	-	-	646314	13.240	3248	0.036	57273	1.085
81	09/12/08	-	-	-	-	787673	16.136	3178	0.035	58394	1.106
82	10/12/08	-	-	2178	0.032	804192	16.474	3339	0.037	60992	1.156

Table O.4 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area			Area	mM	Area
83	11/12/08	-	-	-	-	773444	15.845	2977	0.033	64090	1.214
86	14/12/08	-	-	-	-	772968	15.835	2966	0.033	52033	0.986
89	17/12/08	3749	0.058	-	-	691733	14.171	3283	0.036	50135	0.950
90	18/12/08	6139	0.094	-	-	966711	19.804	4754	0.053	52445	0.994
92	20/12/08	6218	0.096	-	-	888434	18.200	5781	0.064	56543	1.071
94	22/12/08	5152	0.079	-	-	945870	19.377	5384	0.059	60182	1.140
96	24/12/08	5615	0.086	-	-	981779	20.112	3941	0.044	53660	1.017
101	29/12/08	2568	0.039	-	-	865679	17.734	4093	0.045	70779	1.341

Table O.5 OD, cell dry weight and pH values for the *R. capsulatus* YO3 (Hup^-) outdoor CPBR.

Day	Date	OD	Biomass ($gdcw/l_c$)	pH	Cumulative Total Gas Produced (l)
1	16/10/08	0.350	0.163	6.751	0.00
2	17/10/08	0.504	0.235	6.775	0.00
4	19/10/08	0.572	0.266	6.833	0.32
5	20/10/08	0.544	0.253	6.928	0.73
6	21/10/08	0.638	0.297	6.952	1.42
8	23/10/08	0.830	0.386	6.983	3.15
9	24/10/08	0.854	0.398	6.976	4.13
10	25/10/08	0.872	0.406	6.978	4.68
11	26/10/08	0.950	0.442	7.014	5.59
12	27/10/08	1.082	0.504	7.042	6.51
13	28/10/08	1.150	0.535	7.079	6.85
14	29/10/08	1.142	0.532	7.040	8.04
15	30/10/08	1.167	0.543	7.035	9.14
16	31/10/08	1.406	0.655	7.063	9.51
17	01/11/08	1.246	0.580	7.029	9.87
18	02/11/08	1.184	0.551	7.013	9.96
19	03/11/08	1.128	0.525	7.013	10.15
20	04/11/08	1.044	0.486	7.019	10.61
22	06/11/08	0.974	0.453	6.915	11.43
23	07/11/08	0.956	0.445	6.839	11.71
24	08/11/08	0.838	0.390	6.815	12.07
26	10/11/08	0.854	0.398	6.807	12.62
27	11/11/08	0.988	0.460	6.798	13.08
28	12/11/08	0.761	0.354	6.825	13.45
29	13/11/08	0.696	0.324	6.860	13.54
30	14/11/08	0.700	0.326	6.889	13.63
31	15/11/08	0.702	0.327	6.939	13.72
32	17/11/08	0.694	0.323	6.960	14.27
34	19/11/08	0.668	0.311	6.827	14.46
35	20/11/08	0.632	0.294	6.856	14.73
36	21/11/08	0.668	0.311	6.856	15.19
37	22/11/08	0.690	0.321	6.833	15.65
39	24/11/08	0.656	0.305	6.829	16.38
40	25/11/08	0.734	0.342	6.959	17.12
41	26/11/08	0.840	0.391	6.930	18.95
42	27/11/08	0.808	0.376	6.960	20.69

Table O.5 (Continued).

Day	Date	OD	Biomass (gdcw/l_c)	pH	Cumulative Total Gas Produced (l)
43	28/11/08	0.995	0.463	6.950	21.24
44	29/11/08	1.186	0.552	7.027	22.16
46	01/12/08	1.160	0.540	7.054	25.55
47	02/12/08	1.134	0.528	7.028	26.56
49	04/12/08	1.086	0.506	7.040	29.13
50	05/12/08	1.010	0.470	6.997	29.58
52	07/12/08	1.036	0.482	6.993	31.51
53	08/12/08	0.974	0.453	6.961	32.06
54	09/12/08	0.942	0.439	6.989	32.52
55	10/12/08	1.001	0.466	6.997	32.98
56	11/12/08	0.960	0.447	6.988	33.89
59	14/12/08	0.912	0.425	7.032	35.54
60	15/12/08	0.888	0.413	6.999	36.09
61	16/12/08	0.908	0.423	6.982	36.55
62	17/12/08	0.898	0.418	6.826	36.92
63	18/12/08	0.828	0.386	6.815	37.01
65	20/12/08	0.776	0.361	6.950	37.38
67	22/12/08	0.794	0.370	6.897	37.84
69	24/12/08	0.790	0.368	6.924	38.02

Table O.6 HPLC data for the *R. capsulatus* YO3 (Hup$^-$) outdoor CPBR.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	mM	Area
1	16/10/08	2347	0.029	74160	1.287	855059	17.168	-	-	-	-
2	17/10/08	3735	0.046	86422	1.500	783359	15.728	-	-	-	-
5	20/10/08	2628	0.033	79176	1.374	772118	15.503	-	-	-	-
6	21/10/08	3103	0.039	88930	1.543	735780	14.773	-	-	-	-
8	23/10/08	2201	0.027	117509	2.039	595002	11.947	1550	0.039	-	-
9	24/10/08	1249	0.016	126447	2.194	525999	10.561	-	-	-	-
10	25/10/08	1977	0.025	136208	2.364	523123	10.503	-	-	-	-
11	26/10/08	3264	0.041	145116	2.518	394055	7.912	-	-	-	-
12	27/10/08	2609	0.032	156746	2.720	315486	6.334	-	-	-	-
13	28/10/08	86950	1.080	168321	2.921	126669	2.543	-	-	-	-
14	29/10/08	80281	0.997	169974	2.950	65304	1.311	3408	0.085	-	-
15	30/10/08	5276	0.066	166273	2.885	6045	0.121	-	-	-	-
16	31/10/08	2150	0.027	160546	2.786	2833	0.057	-	-	-	-
17	01/11/08	14582	0.181	139362	2.418	1072	0.022	2739	0.068	-	-
18	02/11/08	8844	0.110	131012	2.274	4834	0.097	-	-	-	-
19	03/11/08	3825	0.048	98427	1.708	4870	0.098	-	-	-	-
20	04/11/08	14339	0.178	118670	2.059	7501	0.151	-	-	-	-
22	06/11/08	6301	0.078	117689	2.042	1832	0.037	-	-	-	-
23	07/11/08	8902	0.111	111170	1.929	2703	0.054	-	-	-	-
24	08/11/08	6609	0.082	99559	1.728	1937	0.039	-	-	-	-
26	10/11/08	6731	0.084	95563	1.658	2299	0.046	-	-	-	-
27	11/11/08	3862	0.048	86950	1.509	1429	0.029	-	-	-	-
28	12/11/08	-	-	80281	1.393	-	-	-	-	-	-

Table O.6 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	mM	Area
29	13/11/08	-	-	74810	1.298	-	-	-	-	-	-
30	14/11/08	-	-	75677	1.313	-	-	-	-	-	-
31	15/11/08	-	-	66400	1.152	-	-	-	-	-	-
32	17/11/08	-	-	50361	0.874	174467	3.503	-	-	-	-
33	18/11/08	3703	0.046	66896	1.161	313143	6.287	1863	0.047	-	-
34	19/11/08	2441	0.030	63235	1.097	454796	9.131	3671	0.092	-	-
35	20/11/08	-	-	66744	1.158	576658	11.578	5518	0.138	-	-
36	21/11/08	-	-	76748	1.332	523100	10.503	9159	0.229	-	-
37	22/11/08	-	-	61018	1.059	275641	5.534	6144	0.153	-	-
39	24/11/08	-	-	81614	1.416	430768	8.649	8128	0.203	-	-
40	25/11/08	-	-	100958	1.752	213300	4.283	2355	0.059	-	-
41	26/11/08	1022	0.013	120026	2.083	178815	3.590	-	-	-	-
42	27/11/08	-	-	127894	2.219	227976	4.577	-	-	-	-
43	28/11/08	3661	0.023	365471	4.097	640065	12.851	3757	0.039	9416	0.082
46	01/12/08	11430	0.071	416746	4.672	544147	6.552	5733	0.059	14886	0.130
49	04/12/08	2166	0.014	415296	4.656	546365	6.579	3440	0.035	16017	0.140
52	07/12/08	3770	0.024	534596	5.993	862347	10.384	10790	0.111	27359	0.239
53	08/12/08	5131	0.032	562341	6.304	1089685	13.121	13890	0.143	30161	0.264
54	09/12/08	5145	0.032	534921	5.997	1282510	15.443	15074	0.155	28695	0.251
55	10/12/08	3495	0.022	561279	6.293	1489703	17.938	16509	0.169	31512	0.275
56	11/12/08	4944	0.031	495008	5.550	1429335	17.211	15610	0.160	28510	0.249
60	15/12/08	6469	0.040	588239	6.595	1750866	21.082	28937	0.297	41009	0.358
61	16/12/08	6689	0.042	483106	5.416	1646111	19.821	32982	0.339	35158	0.307

Table O.6 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	Area	mM
62	17/12/08	10372	0.065	466935	5.235	2152108	22.014	35370	0.363	36116	0.316
63	18/12/08	8540	0.053	414205	4.644	2002977	20.488	33331	0.342	31329	0.274
65	20/12/08	8263	0.052	398478	4.467	2002915	20.488	50539	0.519	42494	0.371
69	24/12/08	15694	0.098	446163	5.002	2291244	23.437	51624	0.530	52425	0.458

Table O.7 OD, cell dry weight and pH values for the *R. capsulatus* YO3 (Hup^-) 40 mM/2 mM Ac/Glu fed outdoor CPBR.

Day	Date	OD	Biomass ($gdcw/l_c$)	pH	T(^{0}C)	Cumulative Total Gas Produced (l)
1	18/09/08	0.640	0.298	6.884	28.0	0.00
2	19/09/08	1.712	0.795	7.619	33.0	3.76
3	20/09/08	1.868	0.852	7.507	32.2	8.16
4	21/09/08	1.628	0.755	7.411	32.3	12.84
5	22/09/08	1.548	0.696	7.383	32.0	17.51
6	23/09/08	1.584	0.724	7.394	32.6	21.09
7	24/09/08	1.548	0.716	7.324	32.0	24.30
9	26/09/08	1.488	0.693	7.160	31.5	29.43
10	27/09/08	1.516	0.707	7.171	31.3	32.00
11	28/09/08	1.440	0.697	7.114	31.5	34.75
12	29/09/08	1.428	0.675	7.095	31.2	38.51
13	30/09/08	1.520	0.688	7.137	31.5	41.63
14	01/10/08	1.336	0.629	7.228	31.1	44.83
15	02/10/08	1.320	0.624	7.181	31.5	48.04
16	03/10/08	1.268	0.589	7.138	31.5	51.34
17	04/10/08	1.216	0.583	7.168	31.2	54.55
18	05/10/08	1.220	0.585	7.237	31.5	57.85
19	06/10/08	1.208	0.574	7.158	31.8	61.43
20	07/10/08	1.260	0.558	7.190	31.5	65.46
21	08/10/08	1.164	0.548	7.140	31.7	68.31
23	10/10/08	1.112	0.525	7.112	31.5	70.97
24	11/10/08	1.120	0.521	7.099	30.5	73.62
27	14/10/08	1.032	0.485	7.085	31.0	77.02
29	16/10/08	0.992	0.488	7.092	32.0	81.78
30	17/10/08	1.031	0.473	7.053	31.5	84.54
31	19/10/08	1.012	0.476	7.123	31.3	89.67
32	20/10/08	0.932	0.446	7.050	31.5	92.60
33	21/10/08	0.864	0.421	7.044	30.0	95.45
35	23/10/08	0.944	0.419	7.056	-	100.40

Table O.7 (Continued)

Day	Date	OD	Biomass (gdcw/l_c)	pH	T(^{0}C)	Cumulative Total Gas Produced (l)
36	24/10/08	0.900	0.419	7.010	30.5	103.51
37	25/10/08	0.836	0.389	7.018	32.5	106.82
38	26/10/08	0.806	0.375	7.028	31.5	110.76
39	27/10/08	0.766	0.357	7.030	31.5	113.97
40	28/10/08	0.786	0.366	7.014	32.0	117.27
41	29/10/08	0.750	0.349	7.047	32.5	120.84
42	30/10/08	0.714	0.332	7.036	32.5	123.78
43	31/10/08	0.682	0.318	6.987	32.5	126.16
44	01/11/08	0.718	0.334	7.015	33.7	126.99
45	02/11/08	0.637	0.297	6.992	31.8	127.54
46	03/11/08	0.608	0.283	6.970	31.0	127.90
47	04/11/08	0.552	0.257	6.990	30.5	128.36

Table O.8 HPLC data for the *R.capsulatus* YO3 (Hup$^-$) 40/2 mM Ac/Glu fed CPBR.

Day	Date	Time	Lactic Acid		Formic Acid		Acetic Acid		Butyric Acid	
			Area	mM	Area	mM	Area	mM	mM	Area
1	18/09/08	00.30	-	-	-	-	1183128	23.755	-	-
1	18/09/08	08.00	-	-	-	-	825268	16.570	-	-
1	18/09/08	17.00	4770	0.059	5597	0.097	304484	6.113	-	-
2	19/09/08	10.20	2424	0.030	13510	0.234	212874	4.274	-	-
2	19/09/08	16.50	2345	0.029	15460	0.268	117739	2.364	-	-
3	20/09/08	16.00	1285	0.016	26971	0.468	47088	0.945	-	-
4	21/09/08	16.15	-	-	44433	0.771	28770	0.578	-	-
6	23/09/08	16.00	-	-	82940	1.439	24348	0.489	-	-
7	24/09/08	16.00	-	-	104818	1.819	17390	0.349	-	-
9	26/09/08	16.00	-	-	144028	2.499	3202	0.064	-	-
10	27/09/08	17.30	1336	0.017	163222	2.833	37868	0.760	-	-
11	28/09/08	16.40	-	-	146655	2.545	52015	1.044	-	-
12	29/09/08	15.00	1759	0.022	179179	3.109	53697	1.078	-	-
13	30/09/08	17.30	-	-	183973	3.193	65591	1.317	-	-
14	01/10/08	16.10	-	-	219220	3.804	87670	1.760	-	-
15	02/10/08	16.30	1025	0.013	245826	4.266	103185	2.072	-	-
16	03/10/08	16.00	-	-	235768	4.092	84580	1.698	-	-
17	04/10/08	16.20	-	-	254351	4.414	84757	1.702	-	-
19	06/10/08	16.30	3770	0.047	265748	4.612	88338	1.774	-	-
20	07/10/08	16.30	1372	0.017	248960	4.320	63547	1.276	-	-
21	08/10/08	19.30	1164	0.014	279148	4.844	118481	2.379	-	-

Table O.8 (Continued).

Day	Date	Time	Lactic Acid		Formic Acid		Acetic Acid		Butyric Acid	
			Area	mM	Area	mM	Area	mM	Area	mM
23	10/10/08	16.30	-	-	272354	4.726	45359	0.911	-	-
24	11/10/08	14.45	6582	0.082	368062	6.387	7604	0.153	-	-
27	14/10/08	15.00	3497	0.043	322017	5.588	96087	1.929	-	-
29	16/10/08	16.15	2430	0.030	329907	5.725	142076	2.853	-	-
30	17/10/08	18.15	1337	0.017	332547	5.771	203923	4.094	-	-
31	19/10/08	18.40	3978	0.049	350954	6.090	133811	2.687	-	-
32	20/10/08	14.15	2048	0.025	351908	6.107	168641	3.386	-	-
33	21/10/08	15.50	2527	0.031	353504	6.135	201714	4.050	-	-
35	23/10/08	16.00	1853	0.023	368912	6.402	92801	1.863	-	-
36	24/10/08	15.45	2816	0.035	363269	6.304	171112	3.436	-	-
37	25/10/08	16.00	8586	0.107	355084	6.162	192711	3.869	-	-
38	26/10/08	16.20	7869	0.098	273007	4.738	137433	2.759	-	-
39	27/10/08	16.20	8928	0.111	382836	6.644	238428	4.787	-	-
40	28/10/08	16.00	7909	0.098	341581	5.928	226119	4.540	-	-
41	29/10/08	16.40	9855	0.122	347929	6.038	215599	4.329	-	-
42	30/10/08	17.15	2432	0.030	325452	5.648	79194	1.590	1332	0.026
43	31/10/08	16.10	-	-	363611	6.310	6769	0.136	1255	0.024
44	01/11/08	16.00	-	-	315284	5.471	1399	0.028	-	-
45	02/11/08	16.15	-	-	290622	5.043	-	-	-	-
46	03/11/08	16.25	-	-	250995	4.356	-	-	-	-
47	04/11/08	17.00	-	-	271874	4.718	1091	0.022	-	-

Table O.9 OD, cell dry weight and pH values for the *R. capsulatus* YO3 (Hup^-) 40 mM/2 mM Ac/Glu Fed CPBR with biomass recycle.

Day	Date	OD	gdcw/l_c	pH	Bchla		T(^{0}C)	Cumulative Total Gas Produced (l)
					Abs	$mg_{bch}l/l_c$		
1	12/04/09	0.360	0.168	6.598	0.332	8.55	17.4	0.00
2	13/04/09	0.726	0.338	6.815	0.298	7.68	32.9	5.13
3	14/04/09	0.884	0.412	6.922	0.278	7.16	32.8	9.90
3	14/04/09	1.204	0.561	7.010	0.334	8.60	33.2	13.48
3	14/04/09	0.862	0.401	6.976	0.306	7.88	35.3	15.68
4	15/04/09	1.390	0.647	7.033	0.365	9.40	33.6	16.66
5	16/04/09	1.544	0.719	7.128	0.471	12.13	32.0	19.34
6	17/04/09	1.608	0.749	7.086	0.33	8.50	31.6	21.76
7	18/04/09	1.516	0.706	7.072	0.397	10.23	30.8	24.78
8	19/04/09	1.414	0.658	7.073	0.355	9.14	31.5	27.62
9	20/04/09	1.372	0.639	7.020	0.395	10.17	33.5	30.22
10	21/04/09	1.332	0.620	7.054	0.371	9.56	34.8	32.65
11	22/04/09	1.412	0.657	7.013	0.362	9.32	35.0	37.50
12	23/04/09	1.504	0.700	7.043	0.386	9.94	31.8	41.52
13	24/04/09	1.346	0.627	7.120	0.365	9.40	30.2	43.95
15	26/04/09	1.418	0.660	7.146	0.45	11.59	31.2	46.29
17	28/04/09	1.456	0.678	7.078	0.307	7.91	29.5	49.97
18	29/04/09	1.212	0.564	7.125	0.535	13.78	31.9	52.65
19	30/04/09	1.386	0.645	7.049	0.396	10.20	31.8	54.74
21	02/05/09	1.136	0.529	7.050	0.375	9.66	33.0	57.26
22	03/05/09	1.196	0.557	7.073	0.402	10.36	31.8	59.10
23	04/05/09	1.302	0.606	7.031	0.335	8.63	32.1	61.78
24	05/05/09	1.286	0.599	7.009	0.323	8.32	33.0	9.90
25	06/05/09	1.142	0.532	7.070	0.32	8.24	32.8	13.48

Table O.10 HPLC data for the *R. capsulatus* YO3 (Hup^-) 40 mM/2 mM Ac/Glu fed CPBR with biomass recycle.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid	
		Area	mM	Area	mM	Area	mM
1	13/04/09	-	-	16609	0.255	1649956	32.304
1	13/04/09	-	-	49113	0.754	1561892	30.580
2	14/04/09	1097	0.012	61215	0.940	488368	9.562
3	15/04/09	19590	0.221	224234	3.444	237050	4.641
4	16/04/09	22888	0.259	395234	6.071	212733	4.165
5	17/04/09	10629	0.120	380582	5.846	89443	1.751
6	18/04/09	1772	-	498977	7.664	98453	1.928
7	19/04/09	-	-	537998	8.264	104555	2.047
8	20/04/09	3153	0.036	425319	6.533	105656	2.069
9	21/04/09	1946	0.022	547729	8.413	138797	2.717
10	22/04/09	5654	0.064	543155	8.343	149322	2.924
11	23/04/09	1345	0.015	439785	6.755	142529	2.791
12	24/04/09	1637	0.018	470841	7.232	131393	2.572
14	26/04/09	-	-	459405	7.057	39194	0.767
16	28/04/09	-	-	615440	9.453	1178	0.023
17	29/04/09	-	-	584875	8.984	98819	1.935
18	30/04/09	-	-	599383	9.207	104168	2.039
20	02/05/09	-	-	628859	10.471	54035	1.245
21	03/05/09	-	-	518531	8.634	199824	4.604
22	04/05/09	-	-	568449	9.465	158175	3.644
23	05/05/09	-	-	531151	8.844	156880	3.615

Table O.11 OD, cell dry weight and pH values for the *R. capsulatus* YO3 (Hup^-) 40 mM/3 mM – 40 mM/4 mM Ac/Glu fed CPBR.

Day	Date	OD	gdcw/l_c	pH	Bchla		T(^{0}C)	Cumulative Total Gas Produced (l)
					Abs	mg_{bchl}/l_c		
1	25/03/09	0.498	0.232	6.727	0.105	2.70	24.7	0.00
2	26/03/09	0.554	0.258	6.810	0.131	3.37	31.4	0.00
2	26/03/09	0.608	0.283	6.846	0.213	5.49	31.2	0.00
3	27/03/09	0.672	0.313	6.861	0.180	4.64	31.2	0.18
4	28/03/09	1.030	0.480	7.023	0.287	7.39	29.5	1.93
5	29/03/09	1.164	0.542	7.042	0.300	7.73	32.2	5.59
6	30/03/09	1.294	0.602	7.133	0.342	8.81	32.8	6.97
7	31/03/09	1.656	0.771	7.295	0.244	6.29	35.2	8.34
8	01/04/09	1.580	0.736	7.165	0.28	7.21	33.2	11.28
9	02/04/09	1.544	0.719	7.156	0.318	8.19	30.4	13.84
10	03/04/09	1.453	0.677	7.134	0.285	7.34	34.3	17.79
11	04/04/09	1.384	0.644	7.104	0.285	7.34	30.3	21.18
12	05/04/09	1.380	0.643	7.189	0.266	6.85	32.6	24.94
13	06/04/09	1.234	0.575	7.075	0.276	7.11	31.9	28.97
14	07/04/09	1.210	0.563	7.092	0.321	8.27	29.4	32.46
15	08/04/09	1.022	0.476	7.127	0.176	4.53	29.1	36.12
16	09/04/09	1.054	0.491	7.088	0.287	7.39	31.8	40.07
17	10/04/09	0.966	0.450	7.091	0.332	8.55	30.3	43.83
18	11/04/09	1.036	0.482	7.061	0.315	8.11	31.6	47.59
19	12/04/09	0.914	0.426	7.062	0.340	8.76	32.7	52.44
20	13/04/09	0.872	0.406	7.079	0.342	8.81	32.8	56.02
21	14/04/09	0.876	0.408	6.993	0.319	8.22	33.6	60.24
22	15/04/09	0.842	0.392	6.998	0.295	7.60	33.7	64.82
23	16/04/09	0.840	0.391	7.024	0.336	8.66	31.7	69.50
24	17/04/09	0.832	0.387	7.090	0.335	8.63	29.7	73.81
25	18/04/09	0.814	0.379	7.075	0.336	8.66	30.1	78.03
26	19/04/09	0.820	0.382	7.047	0.316	8.14	31.6	82.43
27	20/04/09	0.804	0.374	7.004	0.365	9.40	33.7	86.37
29	22/04/09	0.842	0.392	7.001	0.327	8.42	33.7	94.07
30	23/04/09	0.870	0.405	7.021	0.38	9.79	32.7	97.83
31	24/04/09	0.824	0.384	7.118	0.326	8.40	31.1	101.31
35	28/04/09	0.922	0.429	7.036	0.301	7.75	30.4	108.74
36	29/04/09	0.896	0.417	6.990	0.405	10.43	33.4	111.95
37	30/04/09	0.946	0.440	7.002	0.355	9.14	30.7	115.98
39	02/05/09	0.864	0.402	7.095	0.333	8.58	32.1	120.20

Table O.12 HPLC data for the *R. capsulatus* YO3 (Hup^-) 40 mM/3 mM – 40 mM/4 mM Ac/Glu fed CPBR.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area			Area	mM	Area
1	26/03/09	-	-	16728	0.257	1716068	33.598	-	-	-	-
2	27/03/09	-	-	22321	0.343	1437047	28.135	1102	0.015	-	-
3	28/03/09	2115	0.024	55334	0.850	1332975	26.098	-	-	-	-
4	29/03/09	-	-	93589	1.438	1046833	20.496	-	-	-	-
6	31/03/09	2808	0.032	205953	3.163	697663	13.659	-	-	-	-
7	01/04/09	2842	0.032	231195	3.551	643737	12.603	-	-	-	-
8	02/04/09	1524	0.017	249235	3.828	575139	11.260	1523	0.021	-	-
10	04/04/09	-	-	233946	3.593	395099	7.735	-	-	-	-
11	05/04/09	-	-	230276	3.537	336401	6.586	3057	0.042	2699	0.034
13	07/04/09	-	-	350990	5.391	327593	6.414	2777	0.039	6270	0.080
14	08/04/09	-	-	398634	6.123	226486	4.434	-	-	7505	0.095
15	09/04/09	-	-	340553	5.231	304526	5.962	-	-	7210	0.092
16	10/04/09	-	-	319392	4.906	356586	6.981	2037	0.028	8366	0.106
17	11/04/09	-	-	497416	7.640	145360	2.846	-	-	6968	0.089
18	12/04/09	-	-	413542	6.352	103017	2.017	-	-	6552	0.083
19	13/04/09	6108	0.069	574964	8.832	192484	3.769	1042	0.014	18070	0.230
20	14/04/09	1310	0.015	422031	6.483	112807	2.209	-	-	18351	0.233
21	15/04/09	5534	0.063	572092	8.788	141604	2.772	-	-	19857	0.253
23	17/04/09	7085	0.080	576186	8.850	56928	1.115	-	-	24040	0.306
24	18/04/09	5564	0.063	538071	8.265	65198	1.276	-	-	24395	0.310

Table O.12 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area			Area	mM	Area
25	19/04/09	-	-	562482	8.640	24676	0.483	-	-	22156	0.282
26	20/04/09	-	-	606436	9.315	23466	0.459	-	-	-	-
27	21/04/09	-	-	588214	9.035	18127	0.355	-	-	-	-
28	22/04/09	-	-	583810	8.967	16122	0.316	-	-	-	-
29	23/04/09	-	-	652514	10.023	9325	0.183	-	-	-	-
30	24/04/09	-	-	574335	8.822	7271	0.142	-	-	-	-
32	26/04/09	-	-	615467	9.454	1048	0.021	-	-	20810	0.265
34	28/04/09	-	-	575807	8.845	16574	0.324	-	-	17685	0.225
35	29/04/09	-	-	627674	9.641	8798	0.172	-	-	17129	0.218
36	30/04/09	-	-	675417	10.375	16671	0.326	-		15625	0.199
38	02/05/09	-	-	639947	9.830	2847	0.056	-	-	-	-
39	03/05/09	-	-	617409	9.484	43390	0.850	-	-	-	-

Table O.13 OD, Cell Dry Weight and pH values for the *R.capsulatus* YO3 (Hup^-) 60 mM/3 mM – 60 mM/4 mM Ac/Glu Fed CPBR.

Day	Date	OD	gdcw/l_c	pH	Bchla		T (^{0}C)	Cumulative Total Gas Produced (l)
					Abs	mg_{bchl}/l_c		
1	21/03/09	0.524	0.244	6.667	0.258	6.65	16.4	0.00
2	22/03/09	1.408	0.656	7.103	0.353	9.09	31.8	2.25
3	23/03/09	1.458	0.679	7.145	0.401	10.33	32.0	4.13
4	24/03/09	1.454	0.677	7.160	0.335	8.63	32.4	6.10
5	25/03/09	1.474	0.686	7.256	0.424	10.92	32.7	7.98
6	26/03/09	1.474	0.686	7.274	0.440	11.33	35.8	10.54
7	27/03/09	1.464	0.682	7.200	0.307	7.91	32.4	12.29
8	28/03/09	1.474	0.686	7.185	0.353	9.09	30.9	14.30
9	29/03/09	1.412	0.657	7.227	0.347	8.94	33.6	16.87
10	30/03/09	1.478	0.688	7.191	0.319	8.22	33.5	18.70
11	31/03/09	1.448	0.674	7.290	0.296	7.62	34.7	21.36
12	01/04/09	1.452	0.676	7.226	0.305	7.86	35.0	23.93
13	02/04/09	1.524	0.710	7.239	0.341	8.78	30.4	26.04
14	03/04/09	1.380	0.643	7.191	0.295	7.60	33.4	28.61
15	04/04/09	1.356	0.631	7.214	0.285	7.34	33.2	30.72
16	05/04/09	1.278	0.595	7.069	0.290	7.47	32.9	33.28
17	06/04/09	1.382	0.643	7.185	0.326	8.40	33.1	35.48
18	07/04/09	1.298	0.604	7.189	0.281	7.24	30.4	37.77
19	08/04/09	1.128	0.525	7.173	0.260	6.70	31.2	39.79
20	09/04/09	1.220	0.568	7.148	0.339	8.73	30.6	41.99
21	10/04/09	1.132	0.527	7.153	0.288	7.42	31.3	45.11
22	11/04/09	1.130	0.526	7.252	0.327	8.42	32.0	48.32
23	12/04/09	1.124	0.523	7.208	0.247	6.36	32.8	2.25
25	14/04/09	1.105	0.514	7.087	0.313	8.06	35.8	4.13
26	15/04/09	1.142	0.532	7.078	0.272	7.01	33.8	6.10
27	16/04/09	1.048	0.488	7.177	0.241	6.21	32	7.98
28	17/04/09	1.308	0.609	7.137	0.38	9.79	31.7	10.54
29	18/04/09	1.184	0.551	7.181	0.341	8.78	30.8	12.29
30	19/04/09	1.174	0.547	7.119	0.331	8.53	32.1	14.30
31	20/04/09	1.210	0.563	7.139	0.357	9.20	33.4	16.87
32	21/04/09	1.172	0.546	7.168	0.417	10.74	34.3	73.81
33	22/04/09	1.164	0.542	7.114	0.362	9.32	33.9	76.56
34	23/04/09	1.266	0.589	7.171	0.344	8.86	33.5	79.95
35	24/04/09	1.228	0.572	7.183	0.304	7.83	32.1	82.33

Table O.13 (Continued).

Day	Date	OD	gdcw/l_c	pH	Bchl		T(^{0}C)	Cumulative Total Gas Produced (l)
					Abs	mg_{bchl}/l_c		
37	26/04/09	1.218	0.567	7.237	0.299	7.70	33.3	87.74
39	28/04/09	1.202	0.560	7.203	0.289	7.44	30.3	91.96
40	29/04/09	1.092	0.508	7.181	0.47	12.11	30.5	93.61
41	30/04/09	1.170	0.545	7.084	0.352	9.07	31.1	96.27
43	02/05/09	1.182	0.550	7.122	0.299	7.70	32.1	100.76
44	03/05/09	1.126	0.524	7.162	0.304	7.83	31.1	103.88

Table O.14 HPLC data for the *R. capsulatus* YO3 (Hup$^-$) 60 mM/3 mM - 60 mM/4 mM Ac/Glu fed CPBR.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	Area	mM
1	21/03/09	-	-	16757	0.257	1325031	25.942	-	-	-	-
2	22/03/09	2190	0.025	36338	0.558	874077	17.113	1713	0.024	-	-
3	23/03/09	-	-	55847	0.858	713325	13.966	-	-	-	-
4	24/03/09	6083	0.069	65397	1.005	690914	13.527	-	-	-	-
6	26/03/09	-	-	93400	1.435	778378	15.240	-	-	-	-
7	27/03/09	2461	0.028	96876	1.488	757483	14.830	-	-	-	-
8	28/03/09	-	-	137072	2.105	877809	17.186	-	-	-	-
9	29/03/09	-	-	148138	2.275	839524	16.437	-	-	-	-
10	30/03/09	-	-	153414	2.356	952124	18.641	-	-	-	-
12	01/04/09	3976	0.045	196569	3.019	1043086	20.422	-	-	-	-
13	02/04/09	4064	0.046	208699	3.206	1118704	21.903	-	-	-	-
14	03/04/09	-	-	205151	3.151	986533	19.315	-	-	-	-
15	04/04/09	-	-	221031	3.395	1009015	19.755	6600	0.073	-	-
17	06/04/09	-	-	302218	4.642	1076110	21.069	25537	0.282	3853	0.073
18	07/04/09	-	-	306782	4.712	1149729	22.510	27091	0.299	4713	0.089
20	09/04/09	-	-	355658	5.463	1205248	23.597	25717	0.284	6711	0.127
21	10/04/09	-	-	353525	5.430	1089561	21.332	24748	0.273	7987	0.151
22	11/04/09	-	-	395111	6.069	1105870	21.651	-	-	-	-
23	12/04/09	-	-	405005	6.221	1063806	20.828	29390	0.325	-	-
24	13/04/09	-	-	352984	5.422	959362	18.783	-	-	-	-
25	14/04/09	-	-	359354	5.520	995976	19.500	-	-	10837	0.205

Table O.14 (Continued).

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid	
		Area	mM	Area	mM	Area	mM	Area	mM	mM	Area
26	15/04/09	-	-	359022	5.515	1124211	22.010	-	-	10746	0.204
28	17/04/09	-	-	421569	6.475	967528	18.943	27060	0.299	5159	0.098
29	18/04/09	-	-	432672	6.646	1079419	21.134	26568	0.293	13275	0.252
30	19/04/09	-	-	453697	6.969	1076776	21.082	-	-	13660	0.259
31	20/04/09	-	-	463357	7.117	1040388	20.369	-	-	13863	0.263
32	21/04/09	-	-	486105	7.467	990474	19.392	-	-	14779	0.280
33	22/04/09	-	-	484176	7.437	900505	17.631	20705	0.229	14484	0.274
34	23/04/09	-	-	490725	7.538	902182	17.663	-	-	14160	0.268
35	24/04/09	-	-	389493	5.983	888288	17.391	26232	0.290	13339	0.253
37	26/04/09	-	-	584137	8.973	761411	14.907	-	-	15747	0.298
39	28/04/09	-	-	654146	10.048	694501	13.597	22072	0.244	16447	0.312
40	29/04/09	-	-	642147	9.864	812842	15.914	-	-	13265	0.251
41	30/04/09	-	-	625432	9.607	715225	14.003	20836	0.230	14507	0.275
43	02/05/09	-	-	674822	10.365	740300	14.494	51140	0.565	14424	0.273
44	03/05/09	-	-	615092	9.448	864404	16.924	54669	0.604	12436	0.236

Table O.15 OD, cell dry weight and pH values for the *R. capsulatus* YO3 (Hup$^-$) 80 mM/4 mM Ac /Glu fed CPBR.

Day	Date	Time	OD	gdcw/l_c	pH	Bchla		T(^{0}C)
						Abs	mg_{bchl}/l_c	
1	21/03/09	0.20	0.668	0.311	6.756	0.400	10.30	16.4
1	21/03/09	0.80	1.112	0.518	6.971	0.410	10.56	32.9
1	21/03/09	14.00	1.300	0.605	7.086	0.460	11.85	31.7
1	21/03/09	20.00	1.392	0.648	7.189	0.466	12.00	32
2	22/03/09	0.20	1.550	0.722	7.379	-	-	32.5
2	22/03/09	0.80	1.744	0.812	7.505	-	-	31.9
2	22/03/09	14.00	1.788	0.832	7.415	0.497	12.80	31.9
3	23/03/09	13.00	1.906	0.887	7.466	0.556	14.32	32
4	24/03/09	12.30	2.133	0.993	7.464	0.588	15.15	32.5
5	25/03/09	14.30	2.250	1.048	7.455	0.589	15.17	31.8
6	26/03/09	17.30	2.258	1.051	7.423	0.605	15.58	32.9
7	27/03/09	14.00	2.210	1.029	7.381	0.402	10.36	31.8
8	28/03/09	13.15	2.190	1.020	7.361	0.553	14.24	30.9
9	29/03/09	19.00	2.258	1.051	7.353	0.521	13.42	32.4
10	30/03/09	14.00	1.978	0.921	7.342	0.516	13.29	32.9
11	31/03/09	14.45	2.168	1.009	7.339	0.528	13.60	34.5
12	01/04/09	16.55	1.914	0.891	7.341	0.502	12.93	30.8

Table O.16 HPLC data for the *R. capsulatus* YO3 (Hup^-) 80 mM/4 mM Ac/Glu fed CPBR.

Day	Date	Lactic Acid		Formic Acid		Acetic Acid		Propionic Acid		Butyric Acid		Glutamic Acid
		Area	mM	Area	mM	Area	mM	Area	mM	mM	Area	mM
1	21/03/09	-	-	17723	0.272	1293678	25.328	-	-	-	-	2.18
2	22/03/09	2780	0.031	45896	0.705	713779	13.975	-	-	-	-	0.76
3	23/03/09	8623	0.031	69217	1.063	595686	11.663	-	-	-	-	0.17
4	24/03/09	-	0.097	67837	1.042	484891	9.493	-	-	-	-	-
5	25/03/09	5032	0.057	93969	1.042	614764	12.036	114420	1.264	3032	0.039	-
6	26/03/09	1763	0.020	107302	1.443	588673	11.525	121724	1.345	5020	0.064	-
8	28/03/09	1243	0.014	159728	1.648	844209	16.528	285952	3.159	7221	0.092	-
9	29/03/09	2316	0.026	207971	2.453	894246	17.508	214967	2.375	-	-	-
10	30/03/09	2987	0.034	267271	3.194	1101793	21.572	228852	2.528	3248	0.041	-
11	31/03/09	1674	0.019	232394	4.105	1056444	20.684	277212	3.062	3246	0.041	0.17
12	01/04/09	3681	0.042	303772	3.570	1224771	23.979	251542	2.779	11338	0.144	-