DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

HYDROGEN GAS PRODUCTION FROM WASTE GROUND WHEAT BY DARK AND LIGHT FERMENTATIONS

by
Hidayet ARGUN

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İZMİR

HYDROGEN GAS PRODUCTION FROM WASTE GROUND WHEAT BY DARK AND LIGHT FERMENTATIONS

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İZMİR

Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"HYDROGEN GAS PRODUCTION FROM WASTE GROUND WHEAT BY DARK AND LIGHT FERMENTATIONS"** completed by **HİDAYET ARGUN** under supervision of **PROF. DR. FİKRET KARGI** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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ABSTRACT

Biological hydrogen gas production from waste ground wheat powder solution (WPS) was investigated using batch and continuous dark and light fermentation systems. Continuous experiments were conducted at optimum operation conditions that were determined during batch experiments. Hydrogen production yield and specific hydrogen production rate were considered as the criteria for performance comparison. Combined fermentation experiments were conducted after completing dark and light fermentation experiments.

In dark fermentation experiments heat pre-treated anaerobic sludge (ANS) was found to be the most effective bacterial culture for hydrogen gas production from boiled WPS compared to pure hydrogen producing anaerobic bacteria. Effects of sludge pre-treatment method, medium composition in terms of C/N, C/P ratios and initial waste wheat powder and biomass concentrations on hydrogen gas production rate and yield were investigated.

Hydrogen production by light fermentation using volatile fatty acid (VFA) containing dark fermentation effluent (DFE) was investigated under different conditions. Hydrogen production performances of pure *Rhodobacter sphaeroides* species (*Rhodobacter sphaeroides* RV, *Rhodobacter sphaeroides* NRLL-1727, *Rhodobacter sphaeroides* DSMZ-158) and their combinations were compared and the mixed culture was found to be the most efficient culture for light fermentation. The optimum initial total VFA, NH_4-N and biomass concentrations and the most suitable light source and intensity were determined.

In combined dark and light fermentations of WPS, the optimum light/dark biomass ratio, initial biomass and waste wheat powder concentrations were determined. Effects of light source, light intensity and lighting regime on hydrogen production rate and yield were investigated.

Finally continuous experiments of combined dark and light fermentation were performed using a 7.6 liter hybrid annular bio-reactor in order to investigate the effects of hydraulic residence time (HRT) on hydrogen gas production rate and yield.

Keywords: Bio-hydrogen, wheat powder solution, dark fermentation, light fermentation, combined fermentation, anaerobic sludge, *Rhodobacter sphaeroides,* batch and continuous operation.

ÖĞÜTÜLMÜŞ ATIK BUĞDAYDAN KARANLIK VE IŞIKLI FERMENTASYONLA HİDROJEN GAZI ÜRETİMİ

ÖZ

Bu çalışmada öğütülmüş atık buğdaydan kesikli ve sürekli işletilen karanlık ve ışıklı fermentasyonla hidrojen gazı üretimi araştırılmıştır. Kesikli deneylerde optimum işletme koşulları belirlendikten sonra sürekli denemelere geçilmiştir. Kesikli deneyler karanlık, ışıklı ve birleşik fermentasyon için yapılmıştır. Performans kıyaslama kriterleri olarak hidrojen üretim verimi ve özgül hidrojen üretim hızı seçilmiştir. Birleşik fermentasyon deneyleri karanlık ve ışıklı fermentasyon deneylerinden sonra yapılmıştır.

Karanlık fermentasyonda, ısıl işleme tabi tutulmuş anaerobik çamurun (ANS) kaynatılmış buğday çözeltisinden (WPS) hidrojen üretiminde hidrojen üretebilen saf anaerobik bakteri kültürlerine kıyasla daha verimli olduğu bulunmuştur. Bu nedenle karanlık fermentasyon deneylerine ısıl işleme tabi tutulmuş anaerobik çamur ile devam edilmiştir. Organizma seçiminden sonra sırasıyla en uygun; ısıl işlem metodu, C/N, C/P oranları ile başlangıç atık buğday tozu ve biyokütle konsantrasyonları saptanmışdır.

Uçucu yağ asitleri içeren karanlık fermentasyon çıkış suyundan ışıklı fermentasyon ile hidrojen üretimi deneyleri yapılmıştır. Öncelikle saf *Rhodobacter sphaeroides* (*Rhodobacter sphaeroides* RV, *Rhodobacter sphaeroides* NRLL-1727, *Rhodobacter sphaeroides* DSMZ-158) kültürleri ve bunların kombinasyonlarından oluşan ışıklı fermentasyon deneyleri yapılmıştır. Üç saf kültürün eşit konsantrasyondaki karışımının en uygun seçenek olduğu bulunmuştur. Optimum başlangıç toplam uçucu yağ asidi, NH_4-N ve biyokütle konsantrasyonları tespit edilip en uygun ışık kaynağı ve ışık şiddeti belirlenmiştir.

Karanlık ve ışıklı birleşik fermentasyonla atık buğday nişastasından hidrojen üretimi degişik şartlarda araştırılmıştır. En yüksek hidrojen üretim hızı ve veriminin sağlandığı koşulları bulmak için sırasıyla en ideal ışıklı/karanlık biyokütle

konsantrasyon oranı, başlangıç biyokütle ve substrat konsantrasyonları, en uygun ışık kaynağı, ışık şiddeti ve aydınlatma periyodu belirlenmiştir.

Son olarak 7.6 litre hacminde iç içe geçmiş halkalı biyoreaktörde hidrolik alıkonma süresinin birleşik fermentasyonda hidrojen üretim ve verimi üzerine etkileri incelenmiştir.

Anahtar Kelimeler: Biyohidrojen, buğday tozu çözeltisi, karanlık fermentasyon, ışıklı fermentasyon, birleşik fermentasyon, anaerobik çamur, *Rhodobacter sphaeroides,* kesikli ve sürekli işletme.

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CHAPTER ONE
INTRODUCTION

1.1 The Problem Statement

Energy is a vital need since the existence of civilization and its demand has increased enormously with the rapidly growing human population. Long years mostly fossil originating fuel reserves (coal, petroleum, gasoline, natural gas etc.) have extensively been used to supply this energy since they were present in nature in huge amounts. But the extensive consumption of those reserves and greenhouse gases (CO_2, NO_x, SO_x etc.) that were emitted to the atmosphere by their combustion have caused serious negative effects to the environment. Therefore, many researchers are trying to find alternative sustainable energy sources that could be used instead of fossil fuels. In this context, hydrogen gas is a nominee as the clean energy carrier of the future.

The main problem of hydrogen is the fact that it is not readily available such as fossil fuels. Nowadays hydrogen production is mainly based on chemical technologies, which require intensive (high temperature and pressure) energy and complex operation. Therefore, new cost effective and environmental friendly technologies need to be developed. Biological hydrogen production offers feasible advantages such as operation under mild conditions and specific conversions. Biohydrogen production from carbohydrate rich renewable resources like waste biomass makes the concept more economical and sustainable. However, biological hydrogen production from such wastes occures with low rates and yields and therefore, needs large reactor volumes.

Hydrogen can be produced biologically by several different methods, (a) bio-photolysis of water by algae, (b) dark Fermentation and (c) photo-fermentation of carbohydrates. The production of hydrogen by algae is a rather slow and light energy requiring process. In addition, released oxygen inhibits the enzymes responsible for hydrogen production. Besides, dark fermentation occurs faster and more efficient by anaerobic microorganisms such as *Clostridium sp.* The end products of anaerobic fermentation are volatile fatty acids containing bound hydrogen which can only be

further decomposed by photo-fermentative bacteria (e.g. *Rhodobacter sp.*). Photo-fermentation alone also needs strict operating conditions and light energy and is costly. Therefore, combining dark and photo-fermentation or applying a sequential process seems to be the most reasonable approach.

The rate and yield of hydrogen production may be improved by using the most suitable cultures, optimization of environmental conditions, designing more effective bio-reactors and eliminating the inhibitors like oxygen, excess NH_4-N and volatile fatty acids during the process.

There is still extensive research continuing in laboratories all over the world trying to find solutions to the above mentioned problems and to adapt new efficient technologies from lab scale to large scale.

1.2 Current Energy Situation and Hydrogen

The gLobal primary energy need today is about 13 TW (or 13 terawatt, which is equal to 13.10^{12} watt) and nearly 75% of it is met by fossil originating resources. (Muradov & Veziroğlu, 2008; Züttel, 2008). According to the U.S. Department of Energy (DOE) 1.5% increase in the annual energy consumption out to 2025 is expected. This is a more rapid increase than projected growth in domestic energy production, resulting to increasing dependence on imported fossil fuels. This trend is almost the same for majority of industrialized countries (Muradov & Veziroğlu, 2008). The population and energy demand of the world during the 20th century increased by a factor of 4 and 12, respectively (Züttel, 2008). According to Table 1.1 it was predicted that the use of conventional fossil fuels will end in the next 50 years and the CO_2 levels will double in the atmosphere. There is a strong need for alternative sustainable energy sources.

An alternative energy supply must sufficiently provide energy output for a long period of time. Renewable sources such as biomass, wind or hydropower contribute nowadays about 5 to 10% of the total energy output and therefore might be better to be called as additive energy sources, instead as long-term and gLobal energy supply option, even their broad availability (Förstner, 1995).

It was reported in the Swiss hydrogen report 2008/2009 that the future energy economy would be based on the renewable energy “sources” i.e. the nuclear fusion in the sun, the nuclear fission in the earth crust and the planet movement. It was stated that the current energy technology is based on energy carriers, however the future energy systems have to work with energy flows (Züttel, 2008).

Table 1.1 Time periods for energy and CO_2 predicaments (Förstner, 1995)

Regional effects of NOx and SO_2 emissions	**Today**
Unfolding of CO_2 problem (doubling of effective atmospheric CO_2 content)	50 years
Meeting energy needs with conventional fosil fuels	50 years
Construction of sufficient and environmentally compatible energy systems	100– 140 years
Meeting energy needs with non-conventional fosil fuels	250 years
Decline of CO_2 loads in the atmosphere by carbonate absorption in oceans	500–1000 years
Decay of radioactive waste storage facilities to values comparable to natural deposits	1000 years
Meeting energy needs with nuclear supplies	15000 years

Hydrogen is considered as the energy carrier of the future, in order to find a solution to the exhaustion of the fossil fuels and their harmful effects to the environment. Hydrogen is not a primary energy source, instead it also serves as a medium through which primary energy sources (such as nuclear and/or solar energy) can be stored, transported and utilized to supply our energy needs (Das & Veziroğlu, 2001).

The energy of hydrogen per kilogram is almost three times bigger than fossil based fuels. Properties regarding combustion and explosion values of hydrogen, methane, propane and gasoline are presented in Table 1.2. About three-quarters of all matter in the universe contains hydrogen which makes it the most abundant element. Hydrogen in the atmosphere and earth’s surface is present in 0.07% and 0.14% respectively. It is the lightest element and represented with the symbol H in the periodic table. The density of hydrogen (0.09 gL^{-1}) is quite smal as compared with the density of air (1.2 gL^{-1}) (Das & Veziroğlu, 2001). At standart temperature and pressure, hydrogen is a colorless, odorless, nonmetallic, tasteless gas (Wikipedia, 2009). When hydrogen is combusted the end product is water which makes hydrogen

a clean and non-polluting fuel. Hydrogen is harmLess to human and the environment when compared with other gaseous fuels such as natural gas (Das & Veziroğlu, 2001).

Table 1.2 Combustion and explosion properties of hydrogen, methane, propane and gasoline. [a]100 kPa and 15.5°C. [b]Average value for a mixture of C_1-C_4 and higher hydrocarbons including benzene. [c]Based on the properties of n-pentane and benzene. [d]Theoretical explosive yields (Züttel, 2008).

	Hydrogen	**Methane**	**Propane**	**Gasoline**
Density of gas at standard conditions [kg/m^3(STP)]	0.084	0.65	2.42	4.4a
Heat of vaporisation [kWh kg^{-1}]	0.1237	0.1416		0.07-0.11
Lower heating value [kWh kg^{-1}]	33.314	13.894	12.875	12.361
Higher heating value[kWh kg^{-1}]	39.389	15.361	14.003	13.333
Thermal conductivity of gas at standard conditions [mW cm^{-1} K^{-1}]	1.897	0.33	0.18	0.112
Diffusion coefficient in air at standard conditions [cm·s^{-1}]	0.61	0.16	0.12	0.05
Flammability limits in air [vol%]	4.0-75	5.3-15	2.1-9.5	1-7.6
Detonability limits in air [vol%]	18.3-59	6.3-13.5		1.1-3.3
Limiting oxygen index [vol%]	5	12.1		11.6[b]
Stoichiometric composition in air [vol%]	29.53	9.48	4.03	1.76
Minimum energy for ignition in air [mJ]	0.02	0.29	0.26	0.24
Autoignition temperature [K]	858	813	760	500-744
Flame temperature in air [K]	2318	2148	2385	2470
Maximum burning velocity in air at Standard conditions [m·s^{-1}]	3.46	0.45	0.47	1.76
Detonation velocity in air at standard conditions [km·s^{-1}]	1.48-2.15	1.4-1.64	1.85	1.4-1.7[c]
Energy[d] of explosion, mass-related [gTNT/g]	24	11	10	10
Energy[d] of explosion, volume-related [gTNT m^3(STP)]	2.02	7.03	20.5	44.2

1.3 Conventional Hydrogen Production Methods

Hydrogen gas is a commercial product that is used in many industrial applications such as for the fixation of nitrogen from the air in the Haber-Bosch ammonia process and for the hydrogenation of fats and oils. Hydrogen gas is also used in large quantities in organic chemistry e.g. in methanol production, in hydro-dealkylation, hydro-cracking, hydro-desulfurization, as a rocket fuel, for welding, for production of hydrochloric acid, for the reduction of metallic ores, and for filling balloons. The conventional production of hydrogen worldwide now amounts to about 5.10^{10} kg per year (Züttel, 2008). At present hydrogen is produced mainly from fossil fuels, biomass and water. The methods of hydrogen production from fossil fuels are (Das & Veziroğlu, 2001):

- Steam reforming of natural gas.
- Thermal cracking of natural gas.
- Partial oxidation of hydrocarbons heavier than naphta.
- Coal gasification.

Methods of hydrogen production from biomass are;

- Pyrolysis or gassification (which produces a mixture of gases, i.e. H_2, CH_4, CO_2, CO, N_2).

Methods of hydrogen production from water are;

- Electrolysis.
- Photolysis.
- Thermochemical process.
- Direct thermal decomposition or thermolysis.
- Biological production.

Among the above stated processes 90% of hydrogen is produced with steam reforming of natural gas or light oil fractions. Other industrial hydrogen production methods are coal gassification and electrolysis. The main energy source for those processes are fossil fuels and occasionally hydroelectricity. Common feature of thermochemical and electrochemical hydrogen generation processes are the need of intensive energy and usually undesirable emissions. Compared with those processes, biological hydrogen production processes operate under mild temperatures and pressures which requiring Less energy. Biological processes are not only environment friendly, but also facilitate the utilization of renewable energy sources which are present in immense amount (Das & Veziroğlu, 2001).

1.4 Biological Processes for Hydrogen Production

A special attention has been given to biological hydrogen production processes about one century ago which became more important after the oil crisis in 1970s (Ni et al., 2006).

Biological hydrogen production processes can be classified as follows (Das & Veziroğlu, 2001; Kapdan & Kargi, 2006):

- Hydrogen production by bio-photolysis.
- Light fermentative hydrogen production.
- Dark fermentative hydrogen production.
- Combined dark and light fermentative hydrogen production.

Enzymes play a major role in biological hydrogen gas production. These enzymes catalyse the chemical reaction $2H^+ + 2e^- \leftrightarrow H_2$. Common feature of hydrogen producing enzymes is that they contain complex metallo-clusters as active sites. Known enzymes responsible for the above mentioned reaction are; nitrogenase, Fe-hydrogenase and NiFe-hydrogenase. While Fe-hydrogenase is used in the biophotolysis, nitrogenase is utilized in photodecomposition of organic componds by photosynthetic bacteria (Manish & Banerjee, 2008). Microorganisms produce

hydrogen for two principle reasons, first to dispose of excess reducing equivalents or as a by product in nitrogen fixation (Kotay & Das, 2008). Light dependent and independent biological hydrogen production processes are summarized in Figure 1.1.

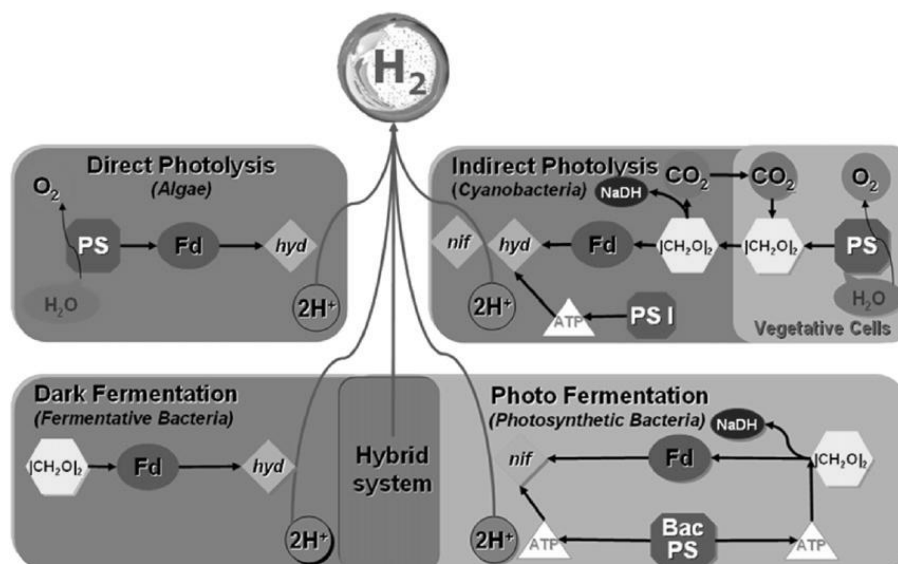


Figure 1.1 Various approaches for biyohydrogen production (Adopted from Kotay, & Das, 2008).

1.4.1 Hydrogen Production by Bio-photolysis

The term biophotolysis is used to denote the splitting of water into hydrogen and oxygen by microorganisms using Light energy. This process is classified as direct and indirect biophotolysis which occurs in green algae and cyanobacteria, respectively. The drawback of biophotolysis is the release of oxygen which negatively effects the hydrogenase and nitrogenase enzymes responsible in hydrogen production. This process is light dependent and slow. Manish & Banerjee (2008) reported rates of 0.07 mmolH_2 h^{-1} L^{-1} and 0.355 mmolH_2 h^{-1} L^{-1} for direct and indirect biophotolysis which are quite low when compared with photo and dark fermentation rates of 145-160 mmolH_2 h^{-1} L^{-1} and 77 mmolH_2 h^{-1} L^{-1} respectively.

During direct photolysis, water is first splitted to hydrogen and oxygen by light energy and hydrogen gas is produced by the reduction of protons. This reaction is catalysed by hydrogenase and nitrogenase enzymes seen in various types of algae. Das & Veziroğlu (2008) reported that hydrogenase activity was oberved in *Scenedesmus obliquus, Chlorococcum littorale, Platymonas subcordiformis* and

Chlorella fusca. However green algae that do not have hydrogenase activity such as *Dunaliella salina* and *Chlorella vulgaris* were also considered by the same author in this category. Hemmes et al. (2003) reported that long term hydrogen could be produced when the released oxygen is removed by passing helium gas from the headspace of the reactor. Also, utilisation of artificial light was found to be more effective than solar energy.

Indirect biophotolysis is the process in cyanobacteria where CO_2 is first fixed from the air to produce carbohydrates as intermediary product and those produced carbohydrates are afterwards utilized in a second reaction for the production of hydrogen and CO_2. In both reactions light energy is used. *Anabaena, Oscillataria, Oscillatoria, Calothrix, Chlamidomonas* are some types of cyanobacteria responsible for hydrogen producion via indirect biophotolysis. Some cyanobacteria have the ability to fix nitrogen from air beside hydrogen production (Das & Veziroğlu, 2001; Kapdan & Kargı, 2006).

1.4.2 Dark Fermentative Hydrogen Production

Dark fermentative hydrogen gas is produced during the heteretrophic decomposition of organic substances e.g. carbohydrates under anaerobic conditions by various types of microorganisms. Spore forming strict anaerobic *Clostridia* species (*C. butyricum, C. thermolacticum, C. pasteurianum, C. Paraputrificum M-21, C. Bifermentans, C. Beijerinkii, C. Acetobutylicum*), facultative enteric bacteria (*Enterobacter aerogenes, Enterobacter cloacae ITT-BY 08*) and some Thermophilic microorganisms (*T. Thermosaccharolyticum, Desulfotomaclucum geothermicum, Thermococcus kodakaraensis*) are most known dark fermentative hydrogen producers (Kapdan & Kargı, 2006). Hydrogen is an intermediary product of methanogenesis and is produced during acidogenesis step, which is later utilized in methanogenic stage by methanogenic bacteria. Therefore, anaerobic sludge from acidogenic phase serves as a potential hydrogen producer only after the elimination of methanogens by pre-treatment. Common pretreatment methods of anaerobic sludge are heat schocking, acid or base treatment, using specific and/or non-specific chemical inhibitors such as chloroform, 2-bromoethanesulsonate (BESA), acetylene,

ethylene, ethane, methyl chloride, methyl fluoride and lumazine for methanogens (Vazquez & Varaldo, 2009). The utilization of pre-treated sludge seems to be more cost effective than using pure cultures. However, production of various volatile organic acids, formation of hydrogen consuming homoacetogens and methanogens with time are main drawbacks.

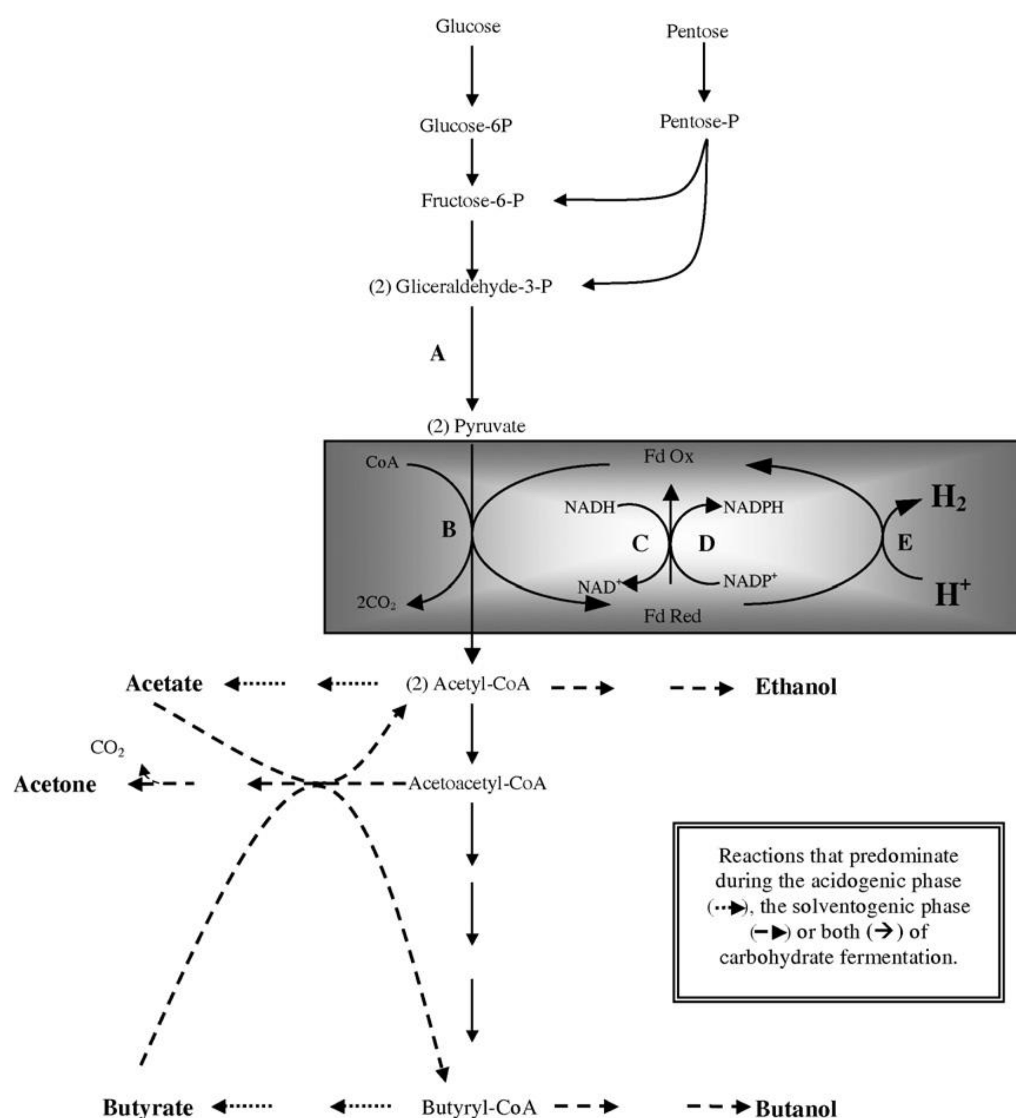
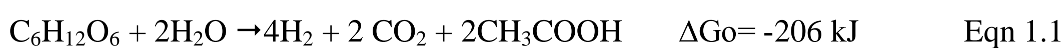


Figure 1.2 Pathway of Clostridial microorganisms during the fermentation of carbohydrates to H_2, CO_2, organic acids and solvents (Adopted from Vazquez & Varaldo, 2009).

Figure 1.2 illustrates the conversion of carbohydrates to H_2, CO_2, organic acids and solvents during dark fermentation for Clostridial microorganisms. The hydrogenase enzyme is the main catalyst in dark fermentative hydrogen gas production. Bacteria conducts these conversions to obtain energy and protons to

serve as electron acceptors that results in formation of molecular hydrogen gas by hydrogenase enzymes (Das & Veziroğlu, 2008) . As shown in Figure 1.2 pyruvate is oxidized to acetyl-CoA after the gLycolytic pathway conversion from glucose to pyruvate. ATP and acetate are produced after this oxidation. In order to oxidize pyruvate, a reduction of ferrodoxin is required. The reduced ferrodoxin can be oxidized by hydrogenase and electrons are released after this step to produce hydrogen. Theoretically a yield of 4 molH_2 mol^{-1}glucose can be produced by dark fermentation if the end product is only acetic acid and if growth/maintenance were negLected. However, it is impossible to obtain that yield in biological dark fermentation since part of the substrate is used for growth and maintenance of cellular material, and other bacterial activities. Also it is not always possible to obtain acetic acid as the only fermentation product. The net reaction of dark fermentative hydrogen production from glucose is given in Eqn 1.1 when only acetic acid is the end product (Manish & Banerjee, 2008). Since the free energy change is negative, bacteria can complete the conversion without any external energy supply.



$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2\ CO_2 + 2CH_3COOH \qquad \Delta Go = -206\ kJ \qquad \text{Eqn 1.1}$$

Other important factors are environmental conditions for an efficient dark fermentation. Optimum pH range for hydrogen production is between 5.0-6.0 (Kapdan & Kargı, 2006). The pH of the medium has a decreasing trend due to formation of volatile organic acids. Temperature optimum varies with the utilized bacteria; for mezophilic bacteria 35-37^oC and for thermophilic bacteria 55^oC are the desirable temperatures. The ORP of the medium has to be kept at anaerobic level (< -150 mV) since the hydrogenase enzyme is very sensitive to oxygen inhibiting its activity. Therefore, chemical reducers such as Na-thioglycolate or L.cysteine.HCl are utilized in order to scavange dissolved oxygen from the media. Passing an inert gas, such as Argon, from the headspace of the fermentation medium is another precaution to remove oxygen. Facultative enteric bacteria are considered as alternative to strict anaerobic bacteria due to their tolerance of small amount of oxygen, and thus eliminating the need for a reducing chemical. Another factor is the sufficient amount of nutrients and trace elements in the media. Those nutrients play important role for growth and other cellular activities.

Many types of wastes that contain cellulose or starch can be utilized as a resource for dark fermentation. When starch is used as a substrate the first step is the acid or enzymatic hydrolysis of biomass to highly concentrated sugar solution which is followed by dark fermentation by acetogenic-anaerobic organisms to produce volatile fatty acids (VFA), hydrogen and CO_2 (Argun et al., 2008a). The rate limiting step is the hydrolysis of starch molecules to carbohydrates. Although dark fermentation has extensively been studied by many researcher, there are still many problems to be solved. However, elimination of light energy, utilization of waste materials and high yields and rates make dark fermentation superior to biophotolysis of water by algae. The produced organic acids that remain in the liquid are potential pollutants and still contain an important amount of bound hydrogen that could be further decomposed. Therefore, the utilization of photo-heteretrophic bacteria capable of fermenting volatile organic acids for hydrogen production is required.

1.4.3 Photo- Fermentative Hydrogen Production

A variety of photosynthetic bacteria or the so called photosynthetic non-sulphur bacteria (PNS) belonging to the *Rhodosprillacae* family such as *Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodobacter sphaeroides-RV, Rhodopseudomonas palustris* have the ability to produce hydrogen gas from different kinds of volatile organic acids by using Light energy. The nitrogenase enzyme is the main catalyst during Light fermentation and requires nitrogen deficient conditions for efficient hydrogen gas production. As shown in Eqn 1.2, theoretically 4 moles of hydrogen can be obtained per mole of acetic acid.

$$CH_3COOH + 2H_2O + \text{'light energy'} \rightarrow 4H_2 + 2\,CO_2 \quad \Delta Go= +208\ kJ \qquad \text{Eqn 1.2}$$

Since the free energy change for this reaction is positive, the reaction does not take place spontaneously without an external energy input. Light energy is utilized as external energy source for this reaction to take place. A description of the related pathway where carbohydrates are metabolized is given in Figure 1.3. Briefly PNS bacteria use organic acids as electron donor and carry those electrons to nitrogenase enzyme by ferrodoxin. The nitrogenase enzyme reduces protons by using ATP and produces hydrogen gas (Manish & Banerjee, 2008).

Figure 1.3 The overall scheme of the carbon source by PNS bacteria during Light fermentation (Adapted from Koku et al., 2002).

PNS bacteria need strict environmental operation conditions during the fermentation period. Optimum pH and temperature range for those bacteria are 6.8-7.5 and 31-36°C respectively (Basak et al., 2007). The type of light source is another factor effecting hydrogen production. PNS prefer light that provides wavelength between 400-1000 nm since maximum light absorbtion was detected at 522 and 860

nm (Akkerman et al., 2002). Besides sun light, various types of artificial light sources such as tungsten, halogen, fluorescent were reported in literature (Koku et al., 2007; Rocha et al., 2002). Light intensity between 6-10 klux was stated to be most suitable value for hydrogen production (Basak et al., 2007). The effect of lighting regime was also compared with continuos illumination (Fascetti et al., 1998; Uyar et al., 2007). Light penetration and PHB (polyhydroxybutyrate) production except hydrogen formation were reported as major problems (Basak et al., 2007; Koku et al., 2007). The presence of any NH_4-N source in the fermentation media terminates or prevents hydrogen formation since nitrogenase produces hydrogen under nitrogen deficiency (Kapdan & Kargi, 2006; Yokoi et al., 1998). However, the use of nitrogen source like Glutamate in certain concentrations was reported in literature for growth (Chen et al., 2007). NH_4-N concentration above 45 mgL^{-1} was found to inhibit hydrogen formation when dark fermentation effluent of wheat powder solution was used as substrate for hydrogen production by PNS bacteria (Argun et al., 2008b). Optimum organic acid concentration was reported to be around 1800-2500 mgL^{-1} (Chen et al., 2007; Laurinavichene et al., 2008; Lee et al., 2007). Since nitrogenase enzyme contains metallo-clusters such as Fe and Mo, addition of such metal ions is quite important for efficient hydrogen production (Kotay & Das, 2008). Many types of wastewater containing organic acids such as dark fermentation effluents can be utilized as substrate for light fermentation which would reduce the cost of raw material.

1.4.4 Combined Dark and Photo-Fermentative Hydrogen Production

Combination of dark and light fermentation is a quite new approach. There are limited number of studies in literature on the subject matter. Fermentation of VFAs from dark fermentation simultaneously improves hydrogen yield per mole of carbohydrate. As stated in Eqn 1.3, it is possible to obtain 12 moles of H_2 per mole glucose when dark and light fermentation are combined if the only VFA is acetic acid. Also, when proper conditions are supplied organic acids resulting from dark fermentation could readily by utilized by PNS bacteria.

$$C_6H_{12}O_6 + 6H_2O + \text{light energy} \rightarrow 12H_2 + 6\,CO_2 \quad \Delta Go = +\,3.2\ kJ \qquad \text{Eqn 1.3}$$

The conversion of glucose to H_2, CO_2 in combined fermentation is illustrated in Figure 1.4. As shown in that pathway light energy is needed for PNS bacteria since conversions from organic acids are not energetically favorable.

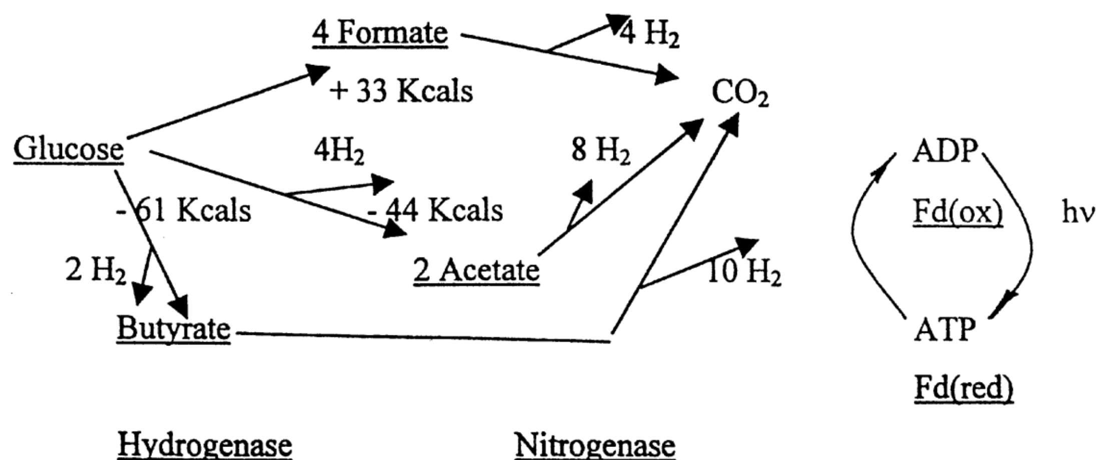


Figure 1.4 Hydrogen production from glucose in combined dark and light fermentation (Adapted from Das & Veziroğlu, 2001).

For an efficient combined fermentation, proper operating conditions such as proper temperature, pH, ORP, light source, light intensity, ligthing regime, biomass ratios of light to dark fermentative microorganisms, addition of required nutrients and trace elements are required. Another important factor is the proper culture selection. Acetic acid forming dark fermentative bacteria with acetate utilizing PNS bacteria seems to be an ideal combination which is almost impossible to obtain. In order to have an effective combined fermentation, PNS bacteria should be adapted to organic acids during the growth phase before inoculation. Different operational modes for combined fermentation such as batch operation in suspended culture, or immobilized PNS bacteria with suspended dark fermentative bacteria were reported in literature (Argun et al., 2009; Asada et al., 2006; Yokoi et al., 1998).

1.5 Waste Wheat as a Potential Resource for Bio-hydrogen Gas Production

Since biological hydrogen gas production occurs under mild conditions, the process does not need large amounts of energy and provides economical advantages

with the utilisation of renewable energy sources. One of those sources is biomass, which is the concentrated energy form of light energy on earth and is produced by photo-synthesis as long as the sun exists. Starch containing waste resources are abandoned in nature as agricultural products (wheat, barley, gruel, rice, corn) and provide a great resource potential for bio-hydrogen gas production. However such wastes need to be pre-treated prior to fermentation. Processing of ligno-cellulosic compounds is more difficult and comprehensive than the processing of starch (Kapdan & Kargı, 2006).

Waste ground wheat from wheat milling process during flour production offers a suitable resource for bio-hydrogen production. This waste is also called as wheat-feed in literature and its composition varies depending on the type of wheat and process. It was reported by Hussy et al. (2007), that the annual world wheat-feed production is about 96 millions of tonnes, which is a considerable amount to select this waste as a resource for bio-hydrogen production. There are many types of wheat all around the world. Table 1.3 represents the characteristics of drum wheat as an example. In general, waste wheat is nutritionally deficient and therefore some nutrients have to be supplemented in certain amounts to the fermentation media (Argun et al., 2008a). Waste wheat should be supplemented by external nitrogen, phosphorous, Fe (II), Mg(II) and minerals for effective bio-hydrogen production by dark anaerobic fermentation.

Table 1.3 Composition of drum wheat (Adopted from United States Department of Agriculture, 2009)

Nutrient	Units	Value per 100 grams
Water	g	10.940
Energy	kcal	339
Energy	kJ	1418
Protein	g	13.680
Total lipid (fat)	g	2.470
Ash	g	1.780
Carbohydrate, by difference	g	71.130
Minerals		
Calcium, Ca	mg	34
Iron, Fe	mg	3.520
Magnesium, Mg	mg	144
Phosphorus, P	mg	508
Potassium, K	mg	431
Sodium, Na	mg	2
Zinc, Zn	mg	4.160
Copper, Cu	mg	0.553
Manganese, Mn	mg	3.012
Selenium, Se	µg	89.400
Vitamins		
Vitamin C, total ascorbic acid	mg	0.0
Thiamin	mg	0.419
Riboflavin	mg	0.121
Niacin	mg	6.738
Pantothenic acid	mg	0.935
Vitamin B-6	mg	0.419
Folate, total	mcg	43
Vitamin B-12	mcg	0.00

1.6 Objectives and Scope of this Study

The objective of this study is to investigate hydrogen production by dark and light fermentation of waste ground wheat and to determine the most suitable operational conditions resulting the highest hydrogen production yield and rate. Batch and continuous operational modes were used for this purpose. Substrate utilization and hydrogen formation were investigated in experimental studies.

Objectives of the study can be summarized as follows:

- To select the most suitable cultures for dark and light fermentations maximizing the hydrogen yield and formation rate.
- To investigate the effects of operating conditions on hydrogen gas production rate and yield in dark anaerobic fermentation in batch systems.
- To investigate the effects of operating conditions on hydrogen gas production rate and yield in light-fermentation process using batch systems.
- To investigate the effects of operation conditions on hydrogen gas production rate and yield in combined dark and light-fermentation process using batch systems.
- To investigate bio-hydrogen production by continuous operation using a hybrid-loop reactor for combined dark and light fermentations and to investigate the effect of hydraulic retention time on hydrogen gas formation yield and rate.

CHAPTER TWO

LITERATURE REVIEW

Various kinds of carbohydrate sources such as glucose, sucrose, molasses, starch, cellulose, food waste, domestic wastewater were used for bio-hydrogen production in dark fermentation (Kapdan & Kargı, 2006; Vazquez & Varaldo, 2009). However, the use of waste materials make the process more attractive in terms of cost reduction and waste minimization. Therefore, many researchers have focused on hydrogen production from carbohydrate containing waste biomass, due to its immense availability and sustainability (Argun et al., 2008a; Arooj et al., 2008; Kapdan & Kargı, 2006; Ni et al., 2006). One of those raw materials is starch, which is a palysaccharide that can abundantly found in plants, corns consequently in agricultural wastes (Kapdan & Kargı, 2006). Another advantage of using starch-containing wastes is that the organic acid containing effluent of dark fermentation can serve as a substrate for light fermentation (Argun et al., 2008b; Lo et al., 2008; Su et al., 2009; Tao et al., 2007a).

The application of different kinds of pure and mixed cultures for dark and light fermentations in batch, repeated batch, continuous and immobilized form for bio-hydrogen gas production were reported in literature (Asada et al., 2006; Das & Veziroğlu, 2001; Fascetti & Todini, 1995; Mohan et al., 2007; Yokoi et al., 1998). Combined and sequential forms of dark and light fermentations were also reported (Argun et al., 2009; Asada et al., 2006; Fang et al., 2006; Lo et al., 2008; Yokoi et al., 1998).

Lee et al. (2008) investigated dark fermentative hydrogen production from cassava starch in batch experiments and used heat-treated sewage sludge as inoculum culture. Maximum hydrogen yield of 231.4 mL H_2 g^{-1} starch was observed at pH=6, T= 37 ^{o}C with 24 gCOD L^{-1} respectively. Also Monod constants were determined as $v_{max,H2}$ =1741 mL h^{-1} L^{-1} and K_s= 14.28 gCOD L^{-1} (Lee et al., 2008).

Dark fermentative hydrogen production from flour industry by-product (wheat-feed) was investigated by Hussy et al. (2007) in a 10 L biorector, inoculated with

sewage sludge fed with 10gL^{-1} wheatfeed at pH=5.5, T=35 ^{o}C in batch and semi-continuous mode. Also, wheat-feed hydrolysate was fermented in continuous mode (HRT=15h). The highest hydrogen yield was 64m^{3} H_2 ton^{-1}dry weight in batch fermentation of wheat-feed.

Yang & Shen (2006) conducted two series of batch dark fermentation experiments with initial pH values of 7 and 8 to produce hydrogen from soluble starch with anaerobic mixed microflora. They investigated the effects of initial $FeSO_4$ and starch concentrations in ranges of 0-4000 mgL^{-1} and 5-40 gL^{-1} respectively. Optimum $FeSO_4$ and starch concentrations were reported as 150 mgL^{-1} and 20 gL^{-1}. Maximum hydrogen yield was 296.2 mLH_2 g^{-1}starch at 150 mg $FeSO_4$ L^{-1} and 10 gL^{-1} starch concentrations (Yang & Shen, 2006).

Hydrogen production with pure *Clostridium butyricum* in batch dark fermentation and batch two-step dark-light fermentations using *Clostridium butyricum* and *Rhodobacter sphaeroides* M-19 from 5gL^{-1} starch was studied by Yokoi et al. (1998). L-cysteine.HCl was used as chemical reducing agent during dark fermentation and incandescent lamp source with 5000 lux illumination was used for light fermentation. Hydrogen yields of dark fermentation, two-step dark and light fermentation and fed-batch operation of dark and light fermentation were 1.9, 3.6 and 6.6 molH_2 mol^{-1} glucose respectively (Yokoi et al., 1998).

Hydrogen production yield of 3.09 molH_2 mol^{-1}glucose was reported by Lo et al. (2008) in a two step continuous dark and light fermentation process where reducing sugar containing starch feed stock was used as raw material. Pure *Clostridium butyricum* CGS2 and *Rhodopsudomonas palustris* WP3-5 were used as dark and light fermentation bacteria. Optimum operational conditions for continuous dark and light fermentation processes were stated as pH= 5.8-6.0, T=37 ^{o}C, HRT=12 h and pH=7, T=35°C, 100Wm^{-2} irradiation, HRT= 48h respectively (Lo et al., 2008).

Yokoi et al. (2001) operated sequential dark and light fermentation processes using mixed cultures of *Clostridium butyricum* and *Enterobacter aerogenes* for dark fermentation and *Rhodobacter sphaeroides* M-19 for light fermentation. Sweet potao starch residue was used as raw material. During dark fermentation substrate was only

supplemented with with 0.1 % polypeptone and without any reducing agent. The effluent of dark fermentation was used as substrate for light fermentation supplemented with 50 µg L^{-1} $Na_2MoO_4.2H_2O$ and 20 mgL^{-1} EDTA. Hydrogen formation yields for dark and light fermentations were reported as 2.4 and 7 $molH_2$ mol^{-1}glucose respectively. A higher hydrogen yield of 7.2 $molH_2$ mol^{-1} glucose was reported by Yokoi et al. (2002) where the same approch was used.

Dark fermentative hydrogen production from hydrolyzed and raw starch were studied by Chen et al. (2008b). Hydrogen production performances of *C. Butyricum* CGS2, *C. Butyricum* CGS5, *C. pasteurianum* CH1, C. *Pasteurianum* CH5 and *C. Pasteurianum* CH7 were compared. It was found that the use of hydrolyzed starch had a pronounced effect on specific hydrogen production rate. Maximum yield and rate were reported to be 118 mL g^{-1}VSS h^{-1} and 1.28 $molH_2$ mol^{-1}glucose by *C. Pasteurianum* CH5. The best starch fermenting strain *C. Butyricum* CGS2 was selected for continuous operation for hydrogen production from hydrolyzed starch. Maximum yield and rate were 2.03 $molH_2.mol^{-1}$ glucose and 534 mL g^{-1}VSS h^{-1}, respectively. It was reported that reduction in HRT from 12h to 2h, considerably increased the hydrogen formation rate, but decreased the yield (Chen et al., 2008b).

Arooj et al. (2008) operated a CSTR fed with corn starch for dark fermentative hydrogen gas production. Mixed community sludge from an anaerobic digester was used as inoculum. Maximum yield of 0.9 $molH_2$ mol^{-1}glucose was reported for HRT=12h. Also, a model to estimate homo-acetogens, relationship with butyrate/acetate ratio at different HRT were developed and used (Arooj et al., 2008).

Krupp & Widmann (2008) observed stable hydrogen gas production with a 30 L working volume reactor fed with waste sugar medium and inoculated with heat-treated anaerobic sludge. Optimum HRT and organic loading rate was reported as 15 h and 14 kg VS m^{-3} d^{-1} respectively (Krupp & Widmann, 2008).

Hydrogen gas was produced by Zurawski et al (2005) under thermophilic conditions (60°C) from glucose, corn starch, potato starch, sugar beet, fodder beet, turnip and potato peels with heat pre-treated anaerobic sludge in batch operation. Although the maximum yield was obtained when glucose was used as substrate (221

mL H_2 g^{-1}VSS), notable hydrogen gas was produced from corn (211 mL H_2 g^{-1}VSS) and potato starch (123 mL H_2 g^{-1}VSS), respectively (Zurawski et al., 2005).

A combination of dark and photo fermentation was studied by Su et al. (2009). *Rhodopseudomonas palustris* was inoculated to the effluent of dark fermentation which was produced by pre-heated mixed microflora. Hydrogen yields and formation rates for dark fermentation were 240.4 mLH_2 g^{-1} starch and 84.4 mL H_2 $L^{-1}h^{-1}$ which were 131.9 mLH_2 g^{-1}starch and 16.4 mLH_2 L^{-1} h^{-1} for light fermentation, respectively. Hydrogen yield dramatically increased from 240.4 mLH_2 g^{-1} starch to 402.3 mLH_2 g^{-1} starch by combining dark and light fermentation (Su et al., 2009).

Ginkel et al. (2009) reported bio-hydrogen gas production from food processing and domestic wastewaters (ww) in batch tests by using 2h baked (100°C) soil inoculums. Overall hydrogen gas productions were stated as 0.7-0.9 LH_2 L^{-1}ww for apple ww, 0.1-2 LH_2 L^{-1}ww for confectioner ww and 2.1-2.8 LH_2 L^{-1}ww for potato ww respectively. Nutrient addition was reported to be beneficial. Hydrogen production and COD removal were correlated. Hydrogen was produced from non-diluted ww with an average yield of 0.1 ± 0.01 LH_2 g^{-1}COD (Ginkel et al., 2009).

Wang et al. (2008b) investigated five different pre-treatment methods of digested sludge (acid, base, heat-shock, aeration and chloroform) in order to select hydrogen producing bacteria from glucose in batch experiments. Heat pretreatment was found to be the most efficient pre-treatment method. Maximal hydrogen production potential, hydrogen production rate, hydrogen production yield were reported as 215.4 mL, 120.4 mL h^{-1}, 221.5 mL g^{-1} glucose respectively (Wang et al., 2008b).

Dark fermentative hydrogen production from dairy wastewater in sequencing batch reactor (SBR) was studied by Mohan et.al. (2007). It was reported that heat and acid pre-treated anaerobic mixed consortia could effectively produce hydrogen (pH=6) depending on the organic loading rate (Mohan et.al., 2007).

Effects of pH on hydrogen production from glucose was investigated using mixed culture of seed sludge in a bioreactor by Fang et. al.(2002). The pH of the media was adjusted from 4.0 to 7.0 with 0.5 increments and steady state was reached in 14 days

for each step. Optimum pH was reported to be 5.5 resulting hydrogen yield of 2.1 ± 0.1 molH_2 mol^{-1}glucose. Hydrogen concentration in the biogas was about 64 ± 0.2 % (Fang et. al., 2002).

Chen et al. (2004) operated a CSTR for dark fermentative hydrogen gas production and determined the kinetic parameters. Sucrose was used as substrate and acclimated sewage sludge as inoculum culture. Dilution rate of 0.125h^{-1} was found to be optimal value resulting in 0.105 molH_2h^{-1}. Maximum specific growth rate, Monod constant (Ks) and the growth yield coefficient were estimated to be 0.172 h^{-1}, 68 mgCOD L^{-1} and 0.1 gg^{-1}, respectively (Chen et al., 2004).

Effects of temperature on dark fermentative hydrogen gas production from glucose by heat treated mixed digested sludge was studied by Wang et al., (2008a) and 40 ^oC was found to be the most suitable temperature.

Lin et al. (2004) stated that the C/N ratio has a significant effect on dark fermentative hydrogen production from sucrose using heat treated sludge. According to their results C/N ratio of 47 resulted in hydrogen production yield and rate of 4.8 molH.mol^{-1} sucrose and 270 mmolH_2 $L^{-1}d^{-1}$ respectively (Lin et al., 2004).

Hydrogen production performance of four different cultures *Clostridium beijerinkii, Rhodobacter sphaeroides,* anaerobic sludge and *Bacillus megatarium* by dark and light fementation were compared by Jeong et al. (2008). Glucose was used as substrate. *Clostridium Beijerinkii* was found to be the most effective culture in terms of hydrogen production yield and rate.

Han & Shin (2004) studied the effects of dilution rate on hydrogen production by dark fermentation from food waste in a leaching-bed reactor by using heat-treated anaerobic sludge. It was reported that dilution rate had a strong effect on hydrogen production, substrate removal and the control of the metabolic pathway from hydrogen- and acid forming to solvent-forming pathway. It was stated that the butyrate/acetate ratio was in agreement with hydrogen production. Optimum dilution rate was found as 4.5 d^{-1} (Han & Shin, 2004).

A high hydrogen production yield of 7.1 molH_2 mol^{-1}glucose was reported by Asada et al. (2006) where a co-culture of *Lactobacillus delbrueckii* NBRC13953 and *Rhodobacter sphaeroides* RV were used for hydrogen production from glucose in batch experiments. Illumination of 0.19 mEinstein m^{-2}s^{-1} by halogen lamp was provided to Roux bottles where the fermentation took place. In this study dark and light microorganisms were co-immobilized in a gel. Optimum ratio for co-immobilization of *Lactobacillus delbrueckii* to *Rhodobacter sphaeroides* RV was 1/5 in OD units (Asada et al., 2006).

Tao et al. (2007a) operated a two step process of dark-and photo-fermentation in batch experiments. Sucrose was used as substrate in dark fermentation by heat treated mixed microflora and the volatile organic acid containing effluent was subjected to photo-fermentation by *Rhodobacter sphaeroides* SH2C. Hydrogen yields of dark and light fermentations were 3.67 molH_2 mol^{-1}glucose and 2.97 molH_2 mol^{-1}glucose, respectively. No organic acids were detected in the effluent of photo-fermentation (Tao et al., 2007a).

Uyar et al. (2007) investigated the effects of light intensity, wavelength and illumination protocol on light fermentative hydrogen production in 55mL gas tight photobioreactors by *Rhodobacter sphaeroides* O.U. 001. Malate and Glutamate were used as carbon and nitrogen sources in a synthetic fermentation medium. Optimum light intensity was found to be 270 Wm^{-2}. The need for an infrared light (750-950nm wavelength) was emphasized. Continuous illumination was found to be more effective than intermittent illumination (Uyar et al., 2007).

Dark fermentation effluent of source selected municipal solid waste was inoculated with *Rhodobacter sphaeroides* RV cells in a 1L chemostat operating at HRT= 25h, T= 30°C, pH= 7.2 and 10 klux illumination with tungsten lamp. The effects of molybden and iron addition were stated to be very important. 100 mL H_2.g^{-1}dry weight.h^{-1} was reported as maximum specific hydrogen production rate (Fascetti et al., 1998).

Inhibiton and consumption behaviour of different organic acids from dark fermentation effluent of starch on growth and hydrogen production by *Rhodobacter*

capsulatus were studied by Laurinavichene et al. (2008). It was reported that butyrate and isobutyrate were only consumed after the depletion of acetate, propionate and lactate. Butanol was found to have the highest inhibition effect (50% inhibition at 50 mM). Hydrogen production was inhibited by 50% at 150 mM phosphate and 50 mM butyrate concentrations (Laurinavichene et al., 2008).

A simulation of the daily sunlight illumination pattern for photo-hydrogen production by *Rhodobacter sphaeroides* RV was investigated by Miyake et al. (1999). Batch indoor and outdoor experiments were carried out using 36 mM sodium succinate as carbon and 10 mM sodium ammonium sulphate as nitrogen source in a synthetic fermentation media. They found that a singLe-step illumination method provided an appropriate simulation of sunlight. Maximum hydrogen production rate was reported as 3.3 $lH_2\ m^{-2}h^{-1}$ (Miyake et al., 1999).

Phototrophic hydrogen production from glucose by pure and co-cultures of *Clostridium butyricum* and *Rhodobacter sphaeroides* was studied by Fang et al. (2006) in batch experiments. Optimum biomass ratio of *Rhodobacter sphaeroides* to *C..butyricum* was found to be 5.9. Hydrogen yield of co-culture was about 110 mL $H_2\ g^{-1}$glucose. Combining dark and light fermentation increased hydrogen production performance compared to individual pure culture fermentations by *C.butyricum* and *Rhodobacter sphaeroides* (Fang et al., 2006).

Nath et al. (2008) studied sequential dark and light fermentation from glucose in batch experiments inoculated with *Eneterobacter colacae* DM 11 and *Rhodobacter sphaeroides* O.U. 001 respectively. Glucose was used as the substrate in dark fermentation and the effluent was used for light fermentation. Hydrogen yields for dark and light fermentation were 3.31 molH_2 mol^{-1}glucose and 1.5-1.72 molH_2 mol^{-1} acetic acid, respectively. Monod kinetic constants were determined as μ_{max}= 0.398 h^{-1} and Ks= 5.509 gL^{-1} (Nath et al., 2008).

Effects of NH_4^+ concentration on suspended, entrapped cultures of *Rhodobacter sphaeroides* and *Clostridium butyricum* and entrappment of those bacteria in form of co-culture were investigated in batch experiments by Zhu et al. (2001) Glucose was used as a substrate (50 mM) and NH_4^+ concentrations were varied between 1-10 mM.

NH_4^+ had a strong inihibitory effect on hydrogen production in suspended culture compared with entarappment. However, hydrogen production recovered after the depletion of NH_4^+ from the media in suspended culture. The highest cumulative hydrogen productions were obtained by suspended *Rhodobacter sphaeroides* (380 mL H_2), entrapped *Rhodobacter sphaeroides* (367mL H_2), entrapped *Clostridium butyricum* (66mL H_2) and entrapped co-culture (106mL H_2) (Zhu et al., 2001).

A rotatable central composite statistical experiment design was used by Chen et al. (2008a) to determine the optimal concentration ranges of acetic and butyric acids for hydrogen production by *Rhodopsudomonas palustris* WP3-5. HAc and HBu concentration ranges resulting optimal hydrogen gas production in photo-fermentation were reported to be 2250-2750 and 2000-3800 mgCOD L^{-1} respectively. The same authors reported the optimal butyric acid, glutamic acid and $FeCl_3$ concentrations as 1832 mgL^{-1}, 607 mgL^{-1} and 54 mgL^{-1} respectively for maximum hydrogen production by *Rhodopseudomonas palustris* in light fermentation (Chen et al., 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experiments with Batch Operation

3.1.1 Experimental Procedure and Medium Composition

3.1.1.1 Batch Dark Fermentation Experiments

Batch dark fermentation experiments were conducted in varying sizes of serum bottles (Isolab-Germany Boro 3.3) equipped with silicone rubber stoppers and screw caps to avoid any gas leakage. The wheat powder (WP) that was used as substrate during the experiments contained approximately 97% (w w^{-1}) starch and gluten, 3.4 mg g^{-1} total nitrogen and 1.72 mg g^{-1} phosphate-P. Unless otherwise specified the WPS was partially hydrolyzed by 1.5 boiling of the WPS prior fermentation. During all sets of experiments argon gas was passed for 3 minutes through the head space of the bottles before incubation in a 37 ^{o}C constant temperature incubator. The initial pH was always adjusted to 7 at the beginning of the dark fermentation experiments and was manually controlled between 6.5-7 with 10 M NaOH solution. Experimental bottles were mixed several times by hand during the fermentations.

3.1.1.1.1 Microbial Culture Selection. Experiments were performed to investigate and compare bio-hydrogen formation capabilities of different anaerobic cultures in pure and mixed forms and to select the most suitable culture maximizing hydrogen formation rate and yield from wheat powder (WP). The tested cultures were *Clostridium acetobutylicum* (NRLL-B 527), *Clostridium butyricum* (DSMZ 10702), *Enterobacter aerogenes* (ATCC 13048), heat treated anaerobic sludge and their mixtures. No external nutrients and minerals were added to the fermentation media containing 10 gL^{-1} WP. Hydrolysis of starch, fermentation of sugar and formation of hydrogen and volatile fatty acids (VFA) by different anaerobic cultures were followed. Gompertz equation was used for correlation of batch dark-fermentation data and to determine the rate and the extent of hydrogen formation for different anaerobic bacteria.

Experiments were carried out in 1 liter serum bottles with an initial working volume of 0.4 liter fermentation broth. Wheat particles were ground and sieved down to -200 mesh size before use in batch experiments. Na-thioglycolate (200 mgL^{-1}) was added to the bottles to adjust the oxidation reduction potential (ORP) to lower than -150 mV. The initial WP and biomass concentrations in all bottles were 10 gL^{-1} and 0.5 gL^{-1}, respectively.

3.1.1.1.2 Selection of Sludge Pre-treatment Method. In order to find an effective pre-treatment method for the enrichment of hydrogen producing spore formers in anaerobic sludge (ANS), a series of batch dark fermentation experiments were conducted for production of hydrogen from wheat powder solution (WPS). Anaerobic sludges from two different anaerobic treatment plants and their mixtures were subjected to repeated heat, chloroform and several combinations of heat-chloroform treatment for selection of the most effective pre-treatment method.

The initial WP and cell concentrations in all bottles were 20 gL^{-1} and 2.375 gL^{-1}, respectively. Phosphate buffer containing 2.8 gL^{-1} K_2HPO_4 and 3.9 gL^{-1} KH_2PO_4 was used in all bottles to control pH around 7.0. Na-thioglycolate (150 mg L^{-1}) was added to the bottles to obtain anaerobic conditions. The following sludge pre-treatment methods were used:

3.1.1.1.2.1 Repeated Heat Treatment. The repeated heat treatment of ANS (10h boiling) was conducted by boiling the sludge twice for 5 h each. The sludges (200 mL) were placed in 500 mL flasks and were boiled on a magnetically stirred hot plate for 10h (2 x 5h). The sludges were rested at room temperature for 17 h between the two heating periods.

3.1.1.1.2.2 Chloroform Treatment. The mixture of sludges was subjected to 0.05% chloroform treatment for 17 h. The sludge (200 mL) was placed in a 500 mL flask and the flask was placed on a shaker at room temperature after chloroform addition.

3.1.1.1.2.3 Combination of Chloroform and Heat Treatment. Chloroform in concentrations of 0.05% and 0.1% were used in combined chemical and heat

treatment of the sludges. Chloroform was added to 500mL flasks containing 200 mL of sludge. The flasks were placed on a shaker at 70 rpm for 17h. Chloroform treated MIX sludges were subjected to heat treatment for 5h as described above. Chloroform treatment was also applied to the MIX sludge after 10 h heat treatment.

3.1.1.1.3 Effects of Wheat Powder Boiling on Hydrogen Production. Effects of WPS boiling on hydrogen gas production in dark fermentation was investigated. Experiments were carried out in 1 liter serum bottles with an initial working volume of 0.290 liter. When investigating the effects of boiling, the WP solution was boiled for 1.5 h for partial hydrolysis of starch before placing into the serum bottles. The results were compared with an unboiled WPS. The initial WP and cell concentrations in all bottles were 10 gL^{-1} and 1.25 gL^{-1}, respectively. L-cysteine.HCl (200 mgL^{-1}) was added to the bottles to obtain anaerobic conditions. The same phosphate buffer mentioned in section 3.1.1.1.2 was used in all bottles to control pH around 7.0. Also 0.1 gL^{-1} $MgSO_4.7H_2O$ and 25 mgL^{-1} $FeSO_4.7H_2O$ were added as chemicals.

3.1.1.1.4 Effects of C/N and C/P Ratios in Dark Fermentation. Effects of external supplementation of nitrogen and phosphorous or C/N and C/P ratios were investigated in batch dark fementation experiments. Variations of hydrogen yield and formation rate with the C/N and C/P ratio were investigated by using a Box-Wilson statistical experiment design approach. The C/N and C/P ratios yielding the highest hydrogen yield and the rate were determined.

Experiments were carried out in 2 liter serum bottles. Wheat particles were ground to -200 mesh size to obtain the wheat powder (WP) and the bottles were filled with 1 liter water containing 20 gL^{-1} WP of -200 mesh. The initial biomass concentration was 0.22 gL^{-1}. Nitrogen and phosphorous contents of the wheat were not considered in C/N and C/P ratios since the nature and availability of N and P compounds in wheat powder were not known. Only the externally added N and P were considered in C/N and C/P ratios. The oxidation reduction potential (ORP) was adjusted to nearly -200 mV by addition of 200 mgL^{-1} Na-thioglycolate. C/N and C/P ratios were adjusted by adding required amount of urea (CON_2H_4) as nitrogen source and KH_2PO_4 as P-source to every bottle yielding the desired ratio.

3.1.1.1.5 Effects of Initial WP and Biomass Concentrations. Effects of initial WP (starch) and biomass concentrations on hydrogen production rate and the yield in dark fermentation of WP solution by the heat treated anaerobic-acidogenic sludge in batch experiments were investigated. The optimum substrate (WP) and biomass concentrations and the biomass/substrate ratio maximizing the hydrogen yield and formation rate were determined.

Experiments were carried out in 0.5 liter serum bottles. Wheat particles were ground and sieved down to -200 mesh size in order to obtain the wheat powder (WP) to be used in the batch experiments. In variable WP experiments, the bottles with different WP concentrations (5-30 gL^{-1}) were inoculated with the heat treated anaerobic sludge to yield 2.6 $g L^{-1}$ initial biomass concentration in 385 mL working volume. In variable biomass concentration experiments, the serum bottles (0.5 liter) contained 0.2 liter media with 20 gL^{-1} WP and were inoculated with different amounts of inoculum culture to yield initial biomass concentrations between 0.5 and 5 gL^{-1}. The oxidation reduction potential (ORP) was adjusted to nearly -200 mV by addition of 100 mgL^{-1} Cysteine.HCl. Since the WP was nitrogen (N) and phosphorous (P) deficient, external N (urea) and P (KH_2PO_4) were added to the bottles to yield C/N = 250 and C/P = 1250 on the basis of added N and P.

3.1.1.2 Batch Experiments of Light Fermentation

All batch light fermentation experiments were conducted in serum bottles with a volume of 310 mL (Isolab-Germany Boro 3.3) equipped with silicone rubber stoppers and screw caps to avoid ant gas leakage. Dark fermentation effluent was used as substrate. During all sets of experiments argon gas was passed for 3 minutes through the head space of the bottles before incubation in a room at 30 ^{o}C constant temperature. Experimental bottles were mixed several times by hand during the fermentations. Control experiments without any inoculation were also performed for each set of experiment. pH, ORP and hydrogen gas measurements were done everyday. Light intensities and irradiation were measured using a light meter LX-1108 LT Lutron (LT Lutron, Taiwan) and an Apogee Pyronometer Sensor- PYR-P 3587 (Apogee, USA), respectively.

3.1.1.2.1 Microbial Culture Selection. Hydrogen production capabilities of different pure *Rhodobacter* species (*R. sphaeroides* NRRL B-1727 (RS-NRLL), *R.sphaeroides* DSMZ-158 (RS-DSMZ) and *R sphaeroides-RV* (RS-RV)) and mixed cultures (RS-NRLL+RS-DSMZ, RS-NRLL+RS-RV, RS-DSMZ+RS-RV and RS-DSMZ+RS-NRLL+RS-RV) were investigated in light fermentation of dark fermentation effluent of wheat powder solution.

Ground wheat (-200 mesh) solution containing 20 gL^{-1} wheat powder (WP) was fermented without illumination for 350 h in order to obtain the effluent containing volatile fatty acids (VFA) to be used in light fermentation experiments. Dark fermentations were carried out by using heat treated anaerobic sludge as the inoculum culture. The effluent contained about 9072 mg L^{-1} TVFA and 210 mgL^{-1} NH_4-N. Since high NH_4-N concentrations above 40 mgL^{-1} is inhibitory for the light fermentation organisms, the dark fermentation effluent was aerated overnight after raising the pH to 10 to remove ammonia from the solution. The effluent was diluted to reduce the TVFA to non-inhibitory levels since TVFA concentration above 2500 mgL^{-1} was reported to be inhibitory for *Rhodobacter* species (*Chen et al.,* 2007; Lee et al., 2007). The initial TVFA and NH_4-N concentrations were nearly 2400 ± 200 and 3.6 mgL^{-1}, respectively in the light fermentation media.

Initial fermentation volume was 245 mL fermentation medium. EDTA and $Na_2MoO_4.2H_2O$ were added to the bottles to yield final concentrations of 20 mgL^{-1} and 50 μgL^{-1} respectively since those chemicals were required by the nitrogenase enzyme of the *Rhodobacter* species (Yokoi H., *et al*., 2002). The initial pH of the media was adjusted to 7.45 and the flasks were inoculated with different *Rhodobacter* species to yield an initial cell concentration of 0.45 gL^{-1} in every bottle. The flasks were illuminated with nearly 5500 lux of fluorescent lamps (Phillips LTD 36W/54, Poland) and light intensities were measured using a light meter (LX-1108 LT Lutron).

When a mixture of *Rhodobacter* cultures were used in the light fermentation experiments all other conditions were the same as the pure culture studies (pH_i = 7.45, T = 30 ^oC, illumination = 5500 lux, TVFA = 2400 mgL^{-1}, NH_4-N = 3.6 mgL^{-1}).

The initial cell concentration was 0.2 gL^{-1} which contained equal amounts of each strain.

3.1.1.2.2 Effects of Initial TVFA and NH_4-N Concentrations. Mixed culture of the three *Rhodobacter* species (RS-NRLL+RS-DSMZ, RS-NRLL+RS-RV, RS-DSMZ+RS-RV and RS-DSMZ+RS-NRLL+RS-RV) were used in experiments investigating the effects of initial TVFA and NH_4-N concentrations on bio-hydrogen formation by light fermentation. The dark fermentation effluent used in this set of experiments contained 7200 mgL^{-1} TVFA and 231 mgL^{-1} NH_4-N, which was diluted with pure water to yield, desired initial TVFA and NH_4-N concentrations. The same experimental conditions were used as that of the previous experiments (pH_i = 7.45, T = 30 ^oC, illumination = 5500 lux, V_o = 190 mL). Initial TVFA and NH_4-N concentrations were varied between 1200-5800 mgL^{-1} and 18-185 mgL^{-1}, respectively. The initial mixed bacteria concentration after inoculation was 0.55 gL^{-1} containing equal amounts of each *Rhodobacter* species.

3.1.1.2.3 Effects of Initial Biomass Concentration. Effects of initial biomass concentration on hydrogen gas production from DFE in light fermentation were investigated by using *Rhodobacter sphaeroides*-RV culture as inoculum. Dark fermentation effluent containing volatile fatty acids was used as substrate in light fermentations. In dark fermentation, 20 gL^{-1} ground wheat powder solution (WPS) was boiled for 1.5h, settled for 16h and the supernatant was filtered to obtain particle-free wheat starch solution. Dark fermentation lasted for about 350 h and the effluent contained 8540 mgL^{-1} TVFA, 140 mgL^{-1} NH_4-N, respectively. In order to remove excess ammonium from the medium, pH of the effluent was adjusted to pH = 10 and aerated overnight to remove ammonia from the solution. The aerated effluent was centrifuged for 30 min at 7000g (8000 rpm) to remove the solids. The supernatant was diluted to reduce the TVFA to nearly 2200 mgL^{-1} since this was reported to be the most suitable VFA concentration for light fermentation (Chen et al., 2007; Lee et al., 2007).

9500 lux light intensity was provided to the outer surface of the bottles using fluorescent lamps (Phillips LTD 36W/54). Initial biomass concentrations was varied between 0.5-5 gL^{-1}. Initial TVFA concentration was adjusted to 2200 mgL^{-1} with a

NH_4-N concentration of 35 mgL^{-1} by diluting the dark fermentation effluent with distilled water. Urea (22 mgL^{-1}) was added to this effluent in order to adjust the initial NH_4-N concentration to 45 mgL^{-1}. The initial fermentation volume was 133 mL containing 0.50 μgL^{-1} $Na_2MoO_4.2H_2O$, 2.8 gL^{-1} K_2HPO_4, 3.9 gL^{-1} KH_2PO_4 as phosphate buffer.

3.1.1.2.4 Effects of Light Source and Light Intensity. Effects of light source and intensity on the rate and the yield of hydrogen production in light fermentation were investigated in batch experiments using dark fermentation effluent of WPS. Pure culture of *R. sphaeroides*-RV were used in batch light fermentation experiments. Tungsten, fluorescent, infrared, halogen lamps and sun light were used as different light sources at a constant light intensity. After selecting the most suitable light source, batch experiments with different light intensities were carried out. The most suitable light source and intensity resulting in the highest rate and yield of hydrogen production were determined.

Dark fermentation effluent containing volatile fatty acids was used as substrate in light fermentations. The same substrate preparation procedure of WPS for dark fermentation as explained in section 3.1.1.2.3 was conducted. Dark fermentation lasted for about 70 h and the effluent contained 3250 ± 30 mgL^{-1} TVFA, 20±10 mgL^{-1} NH_4-N, respectively. In order to remove excess ammonium from the medium, effluent pH was adjusted to pH = 10 and aerated overnight to remove ammonia from the solution. The aerated effluent was centrifuged for 30 min at 7000g (8000 rpm) to remove the solids. The supernatant was diluted to reduce the TVFA to nearly 2000 mgL^{-1}.

Two sets of batch experiments were performed to determine the most suitable light source and light intensity yielding the highest rate and extent of bio-hydrogen formation in light fermentations.

In selection of the most suitable light source, the bottles were illuminated with halogen (Kengo Lighting, PAR 30 75W E27), tungsten (Tung, Philips A55 E27 ES 75 W), Infrared (IR, Philips BR 125 IR 150 W), tungsten and infrared (Tung & IR), and fluorescent lamps (Phillips LTD 36W/54) with 270 Wm^{-2} irradiation since this

was reported to be the most suitable light intensity (Uyar B., *et al.*, 2007). When a combinaton of tungsten and IR lights was used, periodic lighting with 15 min intervals were applied for each light source. The light intensity for sun light varied from 1.5 to 70 klux during daytime. A protective grid was used over the bottles to keep the sun-light illumination around 270 Wm^{-2} at the maximum. Fluorescent lamp illumination was also adjusted to 270 Wm^{-2}. The initial volume, biomass concentration and pH were 250mL, 0.498 gL^{-1} and 7.3, respectively in all bottles except the sun-light illumination (167 mL). Initial TVFA concentration was adjusted to 2100 mgL^{-1} with a NH_4-N concentration of 4 mgL^{-1} before the light fermentation.

After determination of the halogen lamp as the most suitable light source, the intensity of the halogen lamp was varied between 1-10 klux. The inital fermentation volume was 200 mL with pH of 7.3 in this set of experiments. The media contained 1465 mgL^{-1} TVFA, 2.2 mgL^{-1} NH_4-N and 0.632 gL^{-1} biomass concentrations, initially.

During the two sets of experiments 20 mgL^{-1} EDTA and 20 mgL^{-1} $FeSO_4.7H_2O$ (from $FeSO_4.7H_2O$ EDTA complex) were added into the dark fermentation effluents in order to activate the nitrogenase enzymes of *Rhodobacter sphaeroides* -RV.

3.1.1.3 Batch Combined Fermentation Experiments

All batch combined fermentation experiments were conducted in serum bottles with a volume of 310 mL (Isolab-Germany Boro 3.3) equipped with silicone rubber stoppers and screw caps to avoid any gas leakage. The same WP and partial hydrolysis of WPS by boiling prior to fermentation was used as explained in part 3.1.1.1. Phosphate buffer containing 2.8 gL^{-1} K_2HPO_4, 3.9 gL^{-1} KH_2PO_4 was used in order to control the pH around 7. During all sets of experiments argon gas was passed for 3 minutes through the head space of the bottles before incubation in a room at 30 ^oC constant temperature. Experimental bottles were mixed several times by hand during the fermentations. Control experiments without any inoculation were also performed for each set of experiment. pH, ORP and hydrogen gas measurements were done everyday. Light intensities and irradiation were measured using a light

meter LX-1108 LT Lutron (LT Lutron, Taiwan) and an Apogee Pyronometer Sensor-PYR-P 3587 (Apogee, USA), respectively.

3.1.1.3.1 Effects of Dark to Light Biomass Ratio (D/L). Effects of dark to light biomass (bacteria) ratio (D/L) on cumulative hydrogen formation, hydrogen yield and formation rate were investigated by batch combined fermentation experiments. The initial wheat powder (WP) and biomass concentrations were constant at 5 gL^{-1} and 2 gL^{-1}, respectively while the D/L ratio was changed between 1/2 and 1/10 ($w\ w^{-1}$). Only dark and light experiments were also performed for comparison. The bottles were illuminated with flourescence light (9500 lux) from the outer surface. The initial fermentation volume was 133 mL containing 0.50 μgL^{-1} $Na_2MoO_4.2H_2O$, phosphate buffer and 76 mgL^{-1} urea as nitrogen source. Initial pH was adjusted to 7.3. In parallel to the combined fermentation, only dark and light fermentation experiments were also conducted using the same initial cell concentration of 2 gL^{-1} of either the dark or the light fermentation bacteria. The oxidation-reduction potential (ORP) of the medium was around +50 mV at the beginning which decreased to nearly - 200 mV at the end due to formation of VFAs.

3.1.1.3.2 Effects of Initial Substrate and Biomass Concentrations. The major objective of this part of the study was to investigate the effects of the substrate (WP) and cell concentrations on the rate and the yield of hydrogen formation in combined dark-light fermentation and to determine the optimum cell/substrate ratio maximizing hydrogen yield and formation rate. The bottles were illuminated with flourescence light (9500 lux) from the outer surface.

Two sets of batch experiments were carried out to determine the effects of initial wheat powder and the cell concentrations on bio-hydrogen production from ground wheat starch by combined dark and light fermentations. In the first set of experiments, the initial wheat powder concentration was varied between 2.5 and 20 g L^{-1} while the initial cell concentration was constant at X_D = 0.037 gL^{-1} and X_L = 0.374 gL^{-1} yielding X_T = 0.411 gL^{-1} with a D/L ratio of 1/10. The initial fermentation volume was 180 mL. The medium included 10 mg L^{-1} $FeSO_4.7H_2O$ + EDTA complex, 0.75 mgL^{-1} $Na_2MoO_4.2H_2O$ and phosphate buffer. The initial pH of the medium was adjusted to 7.5 with 10M NaOH solution. The initial oxidation

reduction potentials (ORP) were around 60 ± 10 mV which decreased to – 350 ± 20 mV at the end of the fermentation. pH was controlled between 6 and 7 in all experiments by manual pH adjustment

In variable cell concentration experiments the total cell concentration was varied between 0.5 and 5 gL^{-1} while the wheat powder concentration was 5 gL^{-1} with a D/L ratio of 1/7. The initial fermentation volume was 240 mL. The fermentation medium included 132 mgL^{-1} NH_4Cl, 50 μgL^{-1} $Na_2MoO_4.2H_2O$ and phosphate buffer. The pH of the medium was adjusted to 7.5 with 10 M NaOH. The initial ORPs were around 50 ± 10 mV at the beggining which decreased to – 350 ± 50 mV at the end of the fermentation. pH was controlled between 6 and 7 by manual pH adjustment using NaOH.

3.1.1.3.3 Effects of Light Source, Light Intensity and Lighting Regime. Effects of light source, intensity and lighting period on the rate and the yield of hydrogen production in combined dark-light fermentations were investigated in three stes of bact experiments. A mixture of heat treated anaerobic sludge and pure culture of *Rhodobacter sphaeroides*-RV was used as inoculums cultures.

Wheat powder solution (WPS) containing 20 gL^{-1} WP, was boiled for 1.5h, settled for 16h and the supernatant was filtered to remove solids. Fermentation solution contained approximately 4000 mgL^{-1} glucose after partial hydrolysis by boiling. Phosphate buffer, 0.2 g L^{-1} $MgCl_2.2H_20$ and 20 mgL^{-1} $FeSO_4.7H_20$-EDTA complex were added to the supernatant before inoculation. A bottle with sunlight illumination were also used for comparison with the experimental flasks. pH was adjusted to 7-7.5 manually with 5 M NaOH solution twice a day.

In selection of the most suitable light source, the bottles were illuminated with the same light sources and light intensities as explained in section 3.1.1.2.4. The initial volume, biomass concentration and pH were 200mL, 0.517 gL^{-1} (X_D = 0.119 gL^{-1}, X_L = 0.398 gL^{-1}, X_L/X_D = 3.3) and 7.0, respectively. The oxidation-reduction potential (ORP) of the medium was around -125 mV at the beginning and remained almost stable around -150mV during the fermentation period.

In the second set of experiments the light intensity was varied between 1-10 klux using halogen lamp. Inital fermentation volume was 200 mL with pH of 7 containing 0.747 gL^{-1} total biomass concentration (X_D = 0.114 gL^{-1} , X_L = 0.633 gL^{-1}, X_L/X_D = 5.55). The ORP of the medium was around -160 mV at the beginning and decreased to nearly - 400 mV at the end. Due to glucose consumption and VFA formation, medium pH decreased from 7.0 to about 5.8 at the first day of fermentation which was controlled manually around 6.5-7.0 for the whole fermentation period.

The third set of experiments was performed in order to determine the effects of lighting regime on hydrogen production by changing durations of dark/light cycles. Halogen lamp with 10 klux intensity was used and durations of dark/light cycles were changed at six different levels (0.5/0.5, 2/2, 4/4, 6/6, 12/12 h/h and continuous lighting). Initial fermentation volume was 200 mL (except the sunlight experiment contained 80 mL fermentation solution) containing 0.583 gL^{-1} initial biomass (X_D = 0.0728 gL^{-1}, X_L = 0.51 gL^{-1} with X_D/X_L = 1/7) at pH =7.

3.1.2 Organisms

3.1.2.1 Batch Experiments of Dark Fermentation

3.1.2.1.1 Microbial Culture Selection. The anaerobic sludge (ANS) was obtained from the acidogenic phase of anaerobic wastewater treatment plant of PAK MAYA Bakers Yeast Company in Izmir, Turkey. The concentrated sludge was boiled for 5 hours in order to select spore forming and hydrogen producing acidogenic bacteria and to eliminate hydrogen consuming methanogens. The pure anaerobic cultures of *Clostridium butyricum*-DSMZ 10702 (CB) and *Clostridium acetobutylicum*-NRLL-B 527 (CAB) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) and the USDA National Center for Agricultural Utilization Research, Peoria, IL, USA, respectively. Pure culture of *Enterobacter aerogenes* was obtained from American Type Culture Collection, USA (ATCC-13048). The cultures were cultivated in their growth media. The *Clostridium sp* and anaerobic sludge were grown in an incubator shaker at 37 ^{o}C and 75 rpm. *Enterobacter aerogenes* (EA) was grown at 30 ^{o}C and 100 rpm using another incubator shaker.

The heat-treated anaerobic sludge and *Clostridium species* were cultivated in a synthetic media containing glucose (60 gL^{-1}), peptone (10gL^{-1}), yeast extract (0.6 g L^{-1}), $MgSO_4.7H_2O$ (0.25 gL^{-1}), K_2HPO_4 (1 gL^{-1}), KH_2PO_4 (1 gL^{-1}), L-cysteine-HCl.H_2O (0.1gL-1) (Müller, 2002). *Enterobacter aerogenes* was cultivated in a synthetic media containing glucose (10gL^{-1}), yeast extract (1 gL^{-1}), peptone (1 gL^{-1}), $MgSO_4.7H_2O$ (0.25 gL^{-1}), KH_2PO_4 (0.5 gL^{-1}) and urea (0.5 gL^{-1}). Argon gas was passed through the cultivation media before incubation and the cultivation flasks were closed with gas tight rubber stoppers to provide anaerobic conditions. The cultivated organisms were used for inoculation of experimental bottles after three days of incubation.

3.1.2.1.2 Selection of Sludge Pre-treatment Method. The suspended anaerobic sludge was obtained from the acidogenic phase of the anaerobic treatment plant of the PAK MAYA Bakers Yeast Company in Izmir, Turkey (PAK). The granular anaerobic sludge was obtained from the anaerobic wastewater treatment plant of EFES Beer Industry in Izmir, Turkey. Those cultures and their equal volume mixtures were used in batch dark fermentation experiments after pre-treatment. Repeated heat, chloroform and combinations of heat-chloroform treatment methods were applied to the anaerobic sludges in order to select spore forming and hydrogen producing bacteria and to eliminate hydrogen consuming methanogens. The same growth media composition and incubation procedure mentioned in section 3.1.2.1.1 was used. The cultivated organisms were used for inoculation of the experimental bottles after two days of incubation at 37 ^oC.

3.1.2.1.3 Effects of Wheat Powder Boiling on Hydrogen Production. The same anaerobic sludge from PAK MAYA as mentioned in section 3.1.2.1.1 was used. The culture was concentrated and boiled for one hour at pH = 5.9 in order to eliminate hydrogen consuming methanogens and to select spore forming and hydrogen producing acidogenic bacteria. The heat treated anaerobic sludge was cultivated in a synthetic media containing diluted molasses (COD= 20 gL^{-1}) as carbon source, 0.2 gL^{-1} $MgSO_4.7H_2O$, 25 mgL^{-1} $FeSO_4.7H_20$ and L-cysteine-HCl.H_2O (0.2 gL^{-1}). The COD/N/P ratio was adjusted to 100/2/0.5 by the addition of KH_2PO_4 and $(NH_4)_2SO_4$.

3.1.2.1.4 Effects of C/N and C/P Ratios. The anaerobic sludge and the procedure for preparation of the inoculum culture were the same as explained in part 3.1.2.1.1.

3.1.2.1.5 Effects of Initial Substrate and Biomass Concentrations. The organisms and the procedure for preparation of the inoculum culture were the same as explained in part 3.1.2.1.1.

3.1.2.2 Batch Experiments of Light Fermentation

3.1.2.2.1 Microbial Culture Selection. The dark fermentation organisms and the procedure for preparation of the inoculum culture were the same as explained in part 3.1.2.1.1. The cultivated organisms were used for inoculation of the dark fermentation bottles containing 20 gL^{-1} WP after three days of incubation.

The *Rhodobacter* species used in light fermentation experiments were obtained from different sources. *R.sphaeroides* (NRRL B-1727) was obtained from USDA National Center for Agricultural Utilization Research, Peoria, IL, USA. *R. sphaeroides* (DSMZ-158) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in lyophilized form. *R sphaeroides-RV* was obtained from J. Miyake (Personel communication, June 2006). Equal amounts of each culture were mixed together in the inoculum when mixed cultures of the *Rhodobacter* species were prepared

Rhodobacter cultures were first grown on the growth medium which were than transferred to the hydrogen production medium. The following growth media from Fascetti et al. (1998) was modified and used: acetic acid (3 gL^{-1}), butyric acid (3 gL^{-1}), NH_4Cl (0.25 gL^{-1}), yeast extract (0.5 gL^{-1}), K_2HPO_4 (2.8 gL^{-1}), KH_2PO_4 (3.9 gL^{-1}), $Na_2MoO_4.2H_2O$ (0.75 mgL^{-1}), $FeSO_4.7H_2O$ (10 mgL^{-1} from $FeSO_4.7H_2O$ EDTA complex), $MgSO_4.7H_2O$ (0.2 gL^{-1}) at $pH_i = 7.1$. The organisms were grown for three days at 30 ^oC under 3000 lux illumination using an incubator shaker at 150 rpm.

The hydrogen production medium had the same composition as the growth medium with the only exception that 10 mM Na-Glutamate was used instead of 0.25 gL^{-1} NH_4Cl in the production medium. The cells were harvested for 24 hours under

the same conditions as for the growth which were centrifuged and washed with 5 gL^{-1} NaCl solution before inoculation. 50 μgL^{-1} $Na_2MoO_4.2H_2O$ and 20 mgL^{-1} EDTA were added into the dark fermentation effluents in order to activate the nitrogenase enzymes of the *Rhodobacter* species.

3.1.2.2.2 Effects of Initial TVFA and NH_4-N Concentrations. The same microbial cultures with same harvesting procedure presented in part 3.1.2.2.1 were used.

3.1.2.2.3 Effects of Initial Biomass Concentration. Dark fermentation took place by natural microflora originating from the ground waste wheat and the air. No external dark fermentative bacteria was inoculated to the WPS that was fermented in order to obtain the effluent for light fermentation.

Rhodobacter sphaeroides-RV culture was used in light fermentation experiments that were obtained from J. Miyake (personel communication, June 2006). Those bacteria were grown in two steps prior to inoculation. The first growth medium contained 5gL^{-1} glucose, 5 gL^{-1} NaCl, 3 gL^{-1} yeast extract and 16 gL^{-1} nutrient broth with initial pH of 6.8, which was autoclaved at 121^oC for 15 min (Parks, 1997). After two days of incubation cells were harvested by centrifugation with the second modified growth medium from Fascetti et al. (1998), that consisted of 0.8gL^{-1} acetic acid, 0.8gL^{-1} lactic acid, 0.8gL^{-1} butyric acid, 0.3 gL^{-1} yeast extract, 2.8gL^{-1} K_2HPO_4, 3.9 gL^{-1} KH_2PO_4, 0.2 gL^{-1} $MgSO_4.7H_20$, 10mgL^{-1} $FeSO_4.7H_2O$-EDTA complex, 0.75 mgL^{-1} $Na_2MoO_4.2H_2O$ and 10mM Na-Glutamate with initial pH of 7.0. Incubation in the second growth medium took place for one day. Grown cells were harvested by centrifugating with 5gL^{-1} NaCl solution prior inoculation. During whole growth phases incubated flasks were illuminated with 15 klux fluorescent lamps (Phillips LTD 36W/54).

3.1.2.2.4 Effects of Light source and Light Intensity. The dark fermentation organisms and the procedure for preparation of the inoculum culture were the same as explained in part 3.1.2.1.3. The dark fermentation on WPS took place at 37 ^oC and pH= 6.8 in an incubator for 17 hours.

The same *Rhodobacter sphaeroides*-RV culture as explained in section 3.1.2.2.3 was used. The following growth medium from Fascetti et al. (1998) was modified and used for *Rhodobacter sphaeroides*-RV: acetic acid (3 gL^{-1}), butyric acid (3 gL^{-1}), Na-Glutamate (10 mM), yeast extract (0.5 gL^{-1}), K_2HPO_4 (2.8 gL^{-1}), KH_2PO_4 (3.9 gL^{-1}), $MgSO_4.7H_2O$ (0.25 gL^{-1}), $CaCl_2.2H_2O$ (25 mgL^{-1}), EDTA (20 mgL^{-1}), $Na_2MoO_4.2H_2O$ (0.75 mgL^{-1}), $FeSO_4.7H_2O$ (10 mgL^{-1} from $FeSO_4.7H_2O$ EDTA complex), H_3BO_3 (2.8 mgL^{-1}), $MnSO_4.H_2O$ (2.77 mgL^{-1}), $Na_2SO_4.7H_2O$ (0.75 mgL^{-1}), $ZnSO_4.7H_2O$ (0.24 mgL^{-1}), $Cu(NO_3)_2.3H_2O$ (0.04 mgL^{-1}), vitamin B-12 (20 μgL^{-1}) at pH_i = 7.1. The organisms were grown for five days at 30 ^oC under 3000 lux illumination using tungsten lamp.The harvested cells were centrifuged and washed with distilled water before inoculation.

3.1.2.3 Batch Experiments of Combined Fermentation

3.1.2.3.1 Effects of Dark to Light Biomass Ratio (D/L). The anaerobic sludge was obtained from the acidogenic phase of the anaerobic wastewater treatment plant of PAK MAYA Bakers yeast company in Izmir, Turkey. The concentrated sludge was exposed to 10 h heat pre-treatment (90 ^oC) in order to select spore forming and hydrogen producing acidogenic bacteria and to eliminate hydrogen consuming methanogens. The heat treated anaerobic sludge was grown in shake flasks using a gyratory shaker at 37 ^oC and pH = 6.8. The same growth media and procedure as explained in section 3.1.2.1.3 was used for the cultivation of anaerobic sludge. The cultivated cells were centrifugated and resuspended in $MgSO_4.7H_2O$ (0.25 gL^{-1}), K_2HPO_4 (1 gL^{-1}), KH_2PO_4 (1 gL^{-1}) containing water in order to obtain high cell density inoculum culture.

Same *Rhodobacter* species as mentioned in section 3.1.2.2.1 were used in equal amounts. *Rhodobacter* cultures were first grown on their growth medium which were than transferred to the hydrogen production medium. The growth medium was the same as explained in part 3.1.2.2.3. The organisms were grown at 30 ^oC under 3000 lux flourescence light. After two days of incubation, the cells were centrifugated and inoculated into modified hydrogen production medium from Fascetti et al. (1998) containing acetic acid (2gL^{-1}), butyric acid (0.5 gL^{-1}), lactic acid (0.5gL^{-1}), yeast extract (0.5 gL^{-1}), K_2HPO_4 (2.8 gL^{-1}), KH_2PO_4 (3.9 gL^{-1}), Na-Glutamete (1.87 gL^{-}

^{1}), $Na_2MoO_4.2H_2O$ (0.75 mgL^{-1}), $FeSO_4.7H_2O$ (10 mgL^{-1}) from $FeSO_4.7H_2O$ EDTA complex, $MgSO_4.7H_2O$ (0.2 gL) at pH 7.0. The organisms were grown at 30 ^{o}C under 9500 lux flouresance light. After 20h of incubation period the cells were harvested by centrifugation and resuspended in $MgSO_4.7H_2O$ (0.25 gL^{-1}), K_2HPO_4 (1 gL^{-1}), KH_2PO_4 (1 gL^{-1}) containing solution prior to inoculation.

3.1.2.3.2 Effects of Initial Substrate and Biomass Concentrations. The same anaerobic sludge from PAK MAYA Bakers yeast company and cultivation procedure was used as explained in section 3.1.2.1.1. After three days of cultivation the cells were centrifugated and resuspended in $MgSO_4.7H_2O$ (0.25 gL^{-1}), K_2HPO_4 (1 gL^{-1}), KH_2PO_4 (1 gL^{-1}) containing water in order to obtain high cell density inoculum culture. The same *Rhodobacter* species with the same cultivation procedure were used as presented in section 3.1.2.3.1 was applied.

3.1.2.3.3 Effects of Light Source, Light Intensity and Lighting Regime. The same anaerobic sludge from PAK MAYA Bakers yeast company was used with the same cultivation and incubation procedure as mentioned in section 3.1.2.1.3. Light fermentative microorganisms and growth conditions were the same as explained in section 3.1.2.2.4. The harvested cells were centrifuged and washed with distilled water before inoculation.

3.1.3 Analytical Methods

Samples removed from the liquid phase were analyzed for starch (STR), total sugar (TSG), NH_4-N, PO_4-P and total volatile fatty acids (TVFA). For starch analysis, the samples were acidified and boiled for 1.5 hours for complete hydrolysis of starch to sugar and the resulting total sugar concentration was determined by using the acid-phenol method (Dubois et al., 1956). Starch concentration was determined by dividing total sugar concentration by 1.10 since glucose to starch ratio is 1.10 in the hydrolysis reaction of starch.

The samples were centrifuged at 7000g to remove solids from the liquid media and soluble sugar, TVFA and NH_4-N analyses were carried out in clear supernatants. Total sugar content was determined by the acid-phenol spectrometric method

(Dubois et al., 1956). The samples were analyzed in triplicates and results were reproducible within 3% deviation. TVFA analyses were carried out by using analytical kits (Spectroquant, 1.01763. 0001, Merck, Germany) and a PC spectrometer (WTW Photolab S12). NH_4-N and PO_4-P were determined by using analytical kits (Spectroquant NH_4-N 1.14752.0001 and Spectroquant PO_4-P 1.14842.001, Merck, Germany) and a PC spectrometer (WTW Photolab S12). Total nitrogen was measured according to Standard Methods (Greenberg et al., 2005). Individual VFAs in the fermentation effluent were determined by using a high performance liquid chromatograph (HPLC, Agilent 1100) with a prevail organic acid column of 5 µm x 150 mm x 4.6 mm and a UV detector of 220 nm.The carrying medium was 25 mM KH_2PO_4 at pH 2.5 with a flow rate of 1 mL min^{-1}.

Hydrogen gas was sampled from the head space of the bottles everyday by using gas-tight gLass syringes and hydrogen concentration was determined by using a gas chromatograph (Agilent 6890). The GC column was Alltech, Hayesep D 80/100 6” x 1/8” x 085”. Nitrogen gas was used as carrier with a flow rate of 30 mL min^{-1} and the head pressure was 22 psi. Temperatures of the oven, injection, detector, and filament were 35°C, 120°C, 120°C, 140°C, respectively.

The amount of total gas produced was determined by water displacement method everyday using sulfuric acid (2%) and NaCl (10%) containing solution. The cumulative hydrogen gas production was determined by using the following equation (Logan et al., 2002):

$$V_{H2,i} = V_{H2,i-1} + V_W C_{H2,i} + V_{G,i} C_{H2,i} - V_{G,i-1} C_{H2,i-1} \qquad \text{Eqn 3.1}$$

where $V_{H2,i}$ and $V_{H2,i-1}$ are the volumes of cumulative hydrogen (mL) calculated after the ith and the previous measurement; V_W is the total gas volume measured by the water displacement method (mL); $C_{H2,i}$ is the concentration of H_2 gas in the total gas measured by the water displacement method (%); $V_{G,i}$ and $V_{G,i-1}$ are the volumes of the gas in the head space of the bottle for the ith and the previous measurement (mL); $C_{H2,i}$ and $C_{H2,i-1}$ are the percent H_2 in the head space of the bottle for the ith and the previous measurement. The amount of released hydrogen gas and in the head

space of the bottle were measured independently and added up to determine cumulative H_2 formation for every period of sampling.

Biomass concentration in the inoculum was determined according to the Standard Methods by filtering 5 mL sample through a 0.45 μm milipore filter, drying at 105 ^oC and determining the constant dry weight (Greenberg et al., 2005). pH and ORP of the fermentation medium were monitored by using a pH meter and ORP meter with relevant probes (WTW, Germany). Light intensities and irradiation were measured using a light meter LX-1108 LT Lutron (LT Lutron, Taiwan) and an Apogee Pyronometer Sensor- PYR-P 3587 (Apogee, USA), respectively.

3.2 Continuous Combined Fermentation Experiments

3.2.1 Experimental System and Medium Composition

Continuous experiments of combined dark and light fermentation were performed in a 7.632 liter hybrid annular rector depicted in Figure 3.1. This reactor consists of two co-axial cylinders. The inner cylinder is a made of stainless steel which is surrounded with a boro silicate glass cylinder. The fermenter is equipped with gas and liquid sampling ports from the top and bottom respectively. A fluorescent lamp (Phillips LTD 36W/54) is placed inside the metal cylinder in order to supply illumination from the inner side of the fermenter. The operation was started batch-wise with 5 gL^{-1} starch containing boiled WPS inoculated with pure *Clostridium beijerinkii* DSMZ 791 and *Rhodobacter sphaeroides*-RV. The batch operation continued until the starch was almost depleted and then the continuos operation was started by feeding the WPS to the fermenter with a desired flow rate. The volume in the fermentation media was 2.554 liter. The HRT was varied by changing the feed flow rate. The fermenter was manually fed with boiled WPS. Samples were withdrawn from the fermenter every day for pH, ORP, starch, TVFA, NH_4-N, glucose, hydrogen and biomass (total suspended solids) measurements. Argon gas was passed for about 5 minutes from the head space of the fermenter at the beginning and after every sampling in order to keep anaerobic conditions in the reactor. Mixing was performed by two circulation pumps operated one minute per two hours

throughout the day. The outlet of the outer cylinder was fed to the inner cylinder during recirculation. pH of the feed WPS was adjusted to 7.3 and pH of the fermentation media was controlled manually between 7-7.5. The temperature of the media was kept at 32 ± 2°C in a room equipped with an air conditioner. Every continuous operation lasted until the system reached the steady-state with approximately the same starch, glucose, TVFA and hydrogen concentratios in the fermenter. The fermenter was illuminated by fluorescent light from the inner cylinder and with 10 klux halogen lamp source (Kengo Lighting, PAR 30 75W E27), from the outer surface of the fermenter respectively.

In experiments performed for different HRTs every experiment lasted about one week. Continuous experiments were performed at six (6) different HRT levels between 1 and 6 days which were established by changing the feed flow rate while keeping the fermentation volume at 2.554 liter constant level.

The feed solution contained 5 gL^{-1} boiled wheat powder, $MgCl_2.2H_2O$ (0.25 gL^{-1}), K_2HPO_4 (2.8 gL^{-1}), KH_2PO_4 (3.9 gL^{-1}), 20 mgL^{-1} $FeSO_4.7H_2O$ (from $FeSO_4$.7 H_2O EDTA complex), 50 μgL^{-1} $Na_2MoO_4.2H_2O$ and 10 mgL^{-1} $CHCl_3$. Chloroform was added to eliminate methanogenic bacteria from the medium.

3.2.2 Organisms

Cultivation methods and conditions for *Clostridium Beijerinkii* (DSMZ-791) and *Rhodobacter sphaeroides*-RV were the same as presented in sections 3.1.2.1.1 and 3.1.2.2.4 respectively. The initial biomass concentration was 0.729 gL^{-1} (X_D = 0.149 gL^{-1}, X_L = 0.580 gL^{-1}, X_L/X_D = 3.89). Harvested cells were centrifuged and washed with distilled water before inoculation.

3.2.3 Analytical Methods

The analytical methods used were the same as presented in section 3.1.3 .

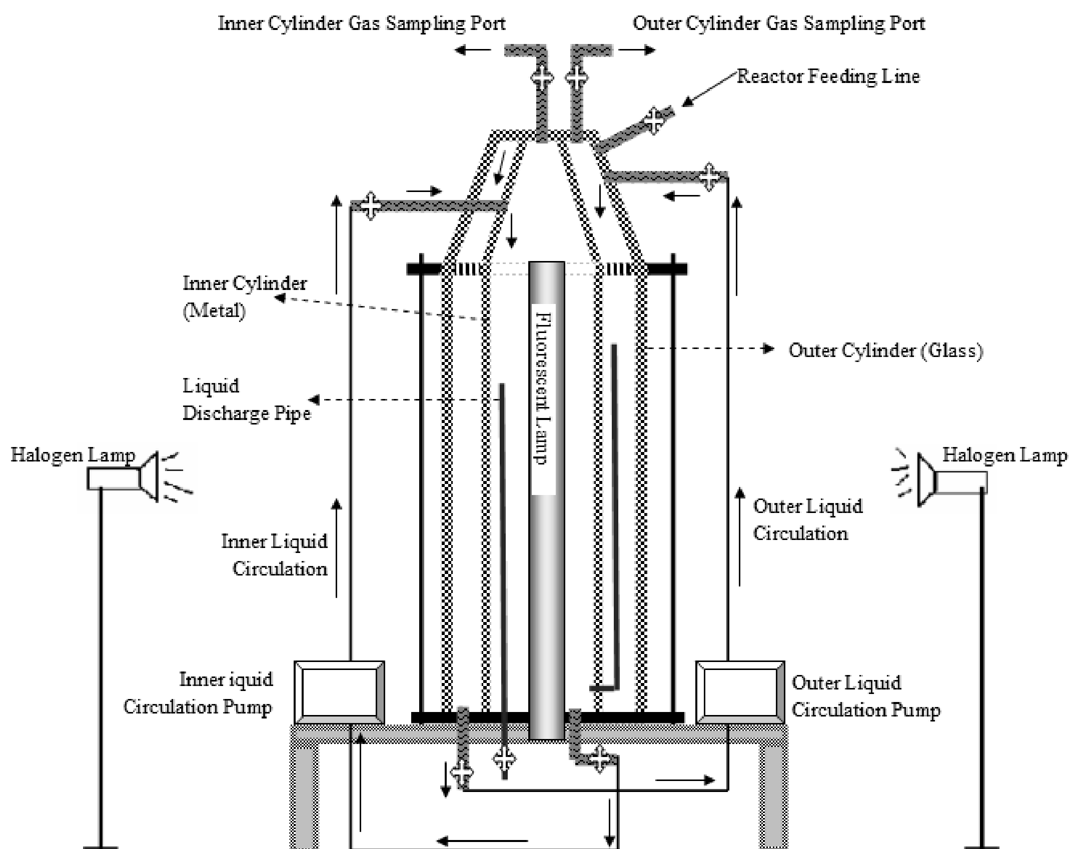


Figure 3.1 A schematic diagram of the hybrid-annular reactor used in continuous combined fermentation experiments.

CHAPTER FOUR

THEORETICAL BACKGROUND

4.1 Batch Experiments

4.1.1 Box-Wilson Statistical Experimental Design

A Box-Wilson statistical experiment design method was used to determine the effects of C/N and C/P ratios on hydrogen yield and production rate in dark fermentation. C/N (X_1) and C/P (X_2) ratios were considered as independent variables; the hydrogen yield (Y_1, mg H_2 g^{-1}starch) and the specific rate of H_2 formation (Y_2, mg H_2 g^{-1} biomass h^{-1}) were considered as the objective functions. N and P are the externally added nutrients exluding the N and P content of the wheat. The C/N and C/P ratios were varied between X_1 = 20-200 and X_2 = 50-1000, respectively. The experiments consisted of four axial and four factorial and a center point totaling 9 experiments at different C/N and C/P ratios. The center point was repeated three times to test the reproducibility of the experiments. Table 4.1 summarizes the list of experiments conducted by using the Box-Wilson statistical experiment design. A control experiment with no inoculation and another experiment with no external N and P addition were performed along with the Box-Wilson design experiments.

Design was codded as -1, -k, 0, +k, +1 and k values were defined by Eqn 4.1

$\pm k$= center point $\pm$ [(max-min)/2 $\sqrt{p}$]   Eqn 4.1

(p=number of variables)

The performance of the system was described by the following response function:

$$Y=\underbrace{b_o+\Sigma b_i*X_i}_{\text{Linear}}+\underbrace{\Sigma b_{ij}*X_i*X_j}_{\text{interaction}}+\underbrace{\Sigma b_{ii}*X_i^2}_{\text{squared}} \qquad i,j=1,2,3.....n \qquad \text{Eqn 4.2}$$

The following quadratic response function was used to correlate the independent variables, X_1 and X_2 with the objective function, Y.

$$Y= b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad \text{Eqn 4.3}$$

The experimental data obtained from the experiments presented in Table 4.1 was used for determination of the coefficients in Eqn 4.3 using a STATISTICA 5 program for regression analysis.

Table 4.1 Experimental conditions of the Box-Wilson statistical design.

Axial points	**X_1**	**X_2**	**Factorial points**	**X_1**	**X_2**
A_1	20	525	F_1	173.60	860.70
A_2	200	525	F_2	173.60	189.31
A_3	110	50	F_3	46.40	860.69
A_4	110	1000	F_4	46.40	189.31
			Centre point	110	525

4.1.2 Calculation Methods for Batch Operation

The generalized gas equation presented in Eqn 4.4 was used to calculate the mole number of cumulative hydrogen.

$$PV = nRT \quad \text{Eqn 4.4}$$

where: n= mmol H_2 gas, P= 1 atm, V_{H2}= Cumulative total hydrogen gas volume (mL), R= 0.082 (L atm / mol K) , T= Temperature in Kelvin (K)

In batch fermentations, cumulative hydrogen versus time data were correlated with the Gompertz equation in Eqn 4.5 and the constants were determined by regression analysis with Statistica 5. The Gompertz equation has the following form (Han & Shin, 2004):

$$H(t) = P \times \exp\left\{-\exp\left[\frac{Rm \times e}{P}(\lambda - t) + 1\right]\right\} \quad \text{Eqn 4.5}$$

where, H is the cumulative hydrogen (mL H_2) at any time t; P is the maximum potential hydrogen formation (mL); R_m is the maximum rate of hydrogen formation

(mL h^{-1}), λ is duration of the lag phase, 'e' is 2.718 and 't' is time (h). The coefficients of the Gompertz equation were determined by regression analysis using the experimental data.

The hydrogen formation yield and specific hydrogen production rate (SHPR) are important parameters indicating the effectiveness of fermentation. The hydrogen formation yield is a thermodynamic phenomenon and expresses the amount of produced hydrogen per mol or gram of consumed substrate. Most yields in literature are reported in molar ratios (mole H_2 mol^{-1} substrate). However, hydrogen yield can also be expressed as the volume of produced hydrogen per mass consumed substrate (mL g^{-1}).

$$Y = \frac{\Delta H_2}{\Delta S} \qquad \text{Eqn 4.6}$$

Where, Y is hydrogen yield (mol H_2/mol substrate) , ΔH_2 is cumulative H_2 (moles or mL) and ΔS is consumed substrate (mol glucose or g starch).

Hydrogen yields in light fermentation (mLH_2 g^{-1}TVFA) were multiplied by TVFA yields in dark fermentation (g TVFA mol^{-1} glucose) to obtain hydrogen yields in light fermentation per mole of glucose (eg, mL H_2 g^{-1}TVFA x gTVFA mol^{-1}glucose = mL H_2 mol^{-1} glucose) which was converted to mol H_2 mol^{-1} glucose at STP. This was added to the dark fermentation yield to obtain the total yield.

The SHPR (mL H_2 g^{-1}biomass h^{-1} at certain temperature and, 1atm) were calculated by using the following equation,

$$R_x = R_m / V_o X_o \qquad \text{Eqn 4.7}$$

where, R_m is the volumetric hydrogen formation rate as calculated from the Gompertz equation (mLH_2 h^{-1}); V_o is the initial volume of the fermentation broth (L) and X_o is the initial biomass concentration (g biomass L^{-1}).

4.1.3 Kinetic Modelling and Estimation of Kinetic Constants

4.1.3.1 Kinetics of dark fermentation

Biohydrogen formation from wheat-starch in dark fermentation can be described by the following reaction

Starch $\longrightarrow$ Glucose + Maltose $\longrightarrow$ VFA + H_2 Eqn 4.8

The first reaction is the enzymatic hydrolysis of starch to glucose and the second is the fermentation of glucose to volatile fatty acids and hydrogen gas. Hydrolysis is the rate limiting step since sugar is readily fermented to VFA and H_2. Therefore, hydrogen formation rate can be related to starch concentration as follows,

$$R_{H2} = \frac{k X_o S_o}{K_s + S_o} \frac{K_I}{K_I + S_o} \qquad \text{Eqn 4.9}$$

where, R_{H2} is the volumetric rate of hydrogen formation (mL H_2 L^{-1} h^{-1}); k is the specific hydrogen formation rate constant (mL H_2 g^{-1} biomass h^{-1}); Xo is the initial biomass concentration (g L^{-1}); So is the initial WP concentration (g L^{-1}); K_s is te saturation constant (g L^{-1}); K_I is the substrate (starch) inhibition constant.

In terms of specific rates Eqn 4.9 can be written as,

$$Rx = \frac{R_{H2}}{X_o} = \frac{k S_o}{K_s + S_o} \frac{K_I}{K_I + S_o} \qquad \text{Eqn 4.10}$$

where Rx is the specific rate of hydrogen formation (mL H_2 g^{-1} biomass h^{-1}).

Batch dark fermentation data for hydrogen production were correlated with eqns 4.9 and 4.10 to determine the kinetic constants.

4.1.3.2 Kinetics of light fermentation

Availability of light intensity affects the performance of light fermentation. In order to quantify the effects of light intensity on hydrogen production rate, the light fermentation data were correlated with the following equations and the constants were determined.

For $I \leq I_m$

$$R = R_m \times (I / Im) \qquad \text{Eqn 4.11}$$

For $I > I_m$, $R = a - b I$

$$\text{or} \quad \frac{R}{R_m} = a - b \frac{I}{I_m} \qquad \text{Eqn 4.12}$$

where R is specific hydrogen production rate ($mLH_2\ g^{-1}biomass\ h^{-1}$); R_m is maximum specific hydrogen production rate ($mLH_2\ g^{-1}biomass\ h^{-1}$) ; I_m is maximum light intensity (lux) at maximum specific hydrogen production rate; k_s is light stimulation constant ($mLH_2\ lux^{-1}\ h^{-1}\ biomass^{-1}$) and k_i is light inhibition constant ($mLH_2\ lux^{-1}\ h^{-1}$ $.biomass^{-1}$)

4.2 Continuos Experiments

4.2.1 Calculation Methods for Continuous Operation

The enrichment of hydrogen content in the headspace of the reactor and produced hydrogen with the release of total gas were considered in calculations of the daily volumetric hydrogen gas production as shown in Eqn 3.1 in section 3.1.3. The mole number of hydrogen, hydrogen production yield and specific hydrogen production rate (SHPR) were calculated as explained in Eqn 4.4, Eqn 4.6 and Eqn 4.7, respectively. The daily starch loading rate (g starch d^{-1}) was calculated by multiplying the initial feeding starch concentration with the flow rate (Q So). The

volumetric hydrogen production rate (VHPR) was calculated by dividing the daily volumetric hydrogen gas production to the volume of the reactor.

CHAPTER FIVE

RESULTS AND DISCUSSION

5.1 Experiments with Batch Operation

5.1.1 Batch Experiments of Dark Fermentation

5.1.1.1 Microbial Culture Selection

Batch dark fermentation experiments were conducted to find the most effective anaerobic culture maximizing the rate and yield of hydrogen formation from WP-starch. Five different anaerobic cultures *Clostridium acetobutylicum* (CAB), *Clostridium butyricum* (CB), *Enterobacter aerogenes* (EA), heat-treated anaerobic sludge (ANS), and their mixtures in equal amounts (MIX) were used in batch experiments under the same conditions. Duration of the experiments was 330 hours.

Figure 5.1 depicts the variation of cumulative hydrogen formation (CHF) with time for different cultures. CHF increased with time for all cultures after a lag phase. The highest CHF was obtained with the heat-treated anaerobic sludge (560 mL) followed by *Enterobacter aerogenes* (546 mL). Hydrogen production by EA was rather slow as compared to the other cultures probably due to low rate of starch hydrolysis. Hydrogen formation by the CAB and MIX cultures was 436 mL and 425 mL, respectively. Contrary to our expectation the mixed culture did not perform as well as the heat-treated ANS. Good performance of the ANS culture was probably due to presence of highly active anaerobic bacteria in the sludge. The lowest CHF (393 mL) was obtained with pure *Clostridum butyricum* (CB) culture.

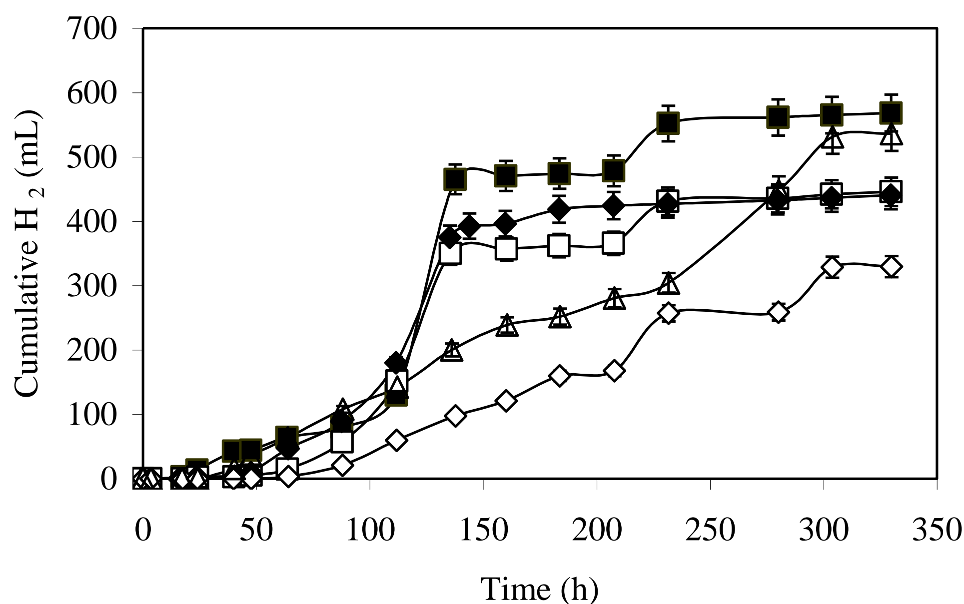


Figure 5.1 Variation of cumulative hydrogen formation with time for different anaerobic cultures (■) ANS, (□) MIX, (♦) CAB, (◊) CB, (Δ) EA.

Cumulative hydrogen data depicted in Figure 5.1 were correlated with the Gompertz equation and the constants were determined by regression analysis as explained in section 4.1.2. Table 5.1 summarizes the Gompertz equation coefficients for different microbial cultures. The highest cumulative hydrogen (558 mL) was obtained with the ANS culture while the highest rate (6.84 mL h^{-1}) was obtained with CAB. Hydrogen production rate (HPR) obtained with ANS (6.42 mLH_2 h^{-1}) was comparable with that obtained with CAB. The lowest lag phase (60.6 h) was obtained with EA culture.

Table 5.1 Gompertz equation coefficients for different anaerobic cultures

Type of Culture	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
ANS	558.50	6.42	82.80	0.96
MIX	425.50	5.55	79.40	0.98
CAB	436.60	6.84	78.30	0.99
CB	393.70	1.64	89.00	0.98
EA	545.80	2.08	60.60	0.98

The initial and final TVFA and starch concentrations are summarized in Table 5.2. Initial TVFA concentrations varied between 300 and 810 mgL^{-1} which were

probably carried by the inoculum culture. Final TVFA concentrations varied between 2600 mgL^{-1} and 4390 mgL^{-1}. The highest final TVFA concentrations were obtained with CAB (4390 mgL^{-1}) and MIX (4110 mgL^{-1}) cultures indicating formation of butyric, propionic and lactic acids rather than acetic acid with low levels of hydrogen formation. Low levels of VFA formation with ANS and EA cultures indicated high levels of hydrogen formation with relatively low VFA levels (ie, high H_2/VFA ratio). High levels of hydrogen formation resulted in low levels of VFA formation due to a shift in metabolic pathway depending on the type of bacteria present in the culture. The final starch concentrations varied between 420 and 905 mgL^{-1} and more than 90% starch was hydrolysed with all bacterial cultures tested except the CB culture. Starch hydrolysis with the CB was the lowest (88.5%). Apparently, all anaerobic cultures hydrolysed starch effectively. However, the difference was at the fermentation step (conversion of glucose to VFA and H_2) where the H_2/ TVFA ratio changed depending on the type and composition of the cultures. H_2/ TVFA ratio (280mL H_2/ g VFA formed) was the highest with the ANS indicating effectiveness of bacteria present in the heat-treated anaerobic sludge for hydrogen formation.

Table 5.2 Initial and final TVFA and starch concentrations for different anaerobic cultures

Type of culture	**$TVFA_o$ (mgL^{-1})**	**$TVFA_F$ (mgL^{-1})**	**$Starch_o$ (mgL^{-1})**	**$Starch_F$ (mgL^{-1})**	**% Starch consumption**
ANS	810	2810	6800	420	93.80
MIX	618	4110	8820	425	95.20
CAB	384	4390	9230	450	95.10
CB	435	2660	7850	905	88.50
EA	294	3150	9330	895	90.40

Table 5.3 summarizes hydrogen yields (HY) and specific hydrogen production rates (SHPR) for all cultures. ANS had the highest hydrogen yield (1.14 mol H_2 mol^{-1} glucose) followed by EA (1.04 mol H_2 mol^{-1} glucose). Hydrogen yields with the other cultures were between 0.8 and 0.9 mol H_2 mol^{-1} glucose. However, the SHPR for CAB was the highest (34.2 mL H_2 g^{-1} cells h^{-1}) followed by the ANS (32.1 mLH_2 g^{-1}cells h^{-1}) and the MIX cultures (27.8 mLH_2 g^{-1}cells h^{-1}). The SHPRs of CB and EA were lower as compared to the other cultures. Mixing all cultures did not improve hydrogen yield as expected since the contribution of ANS was low in the mixture.

Table 5.3 Hydrogen yields and SHPR for different anaerobic cultures

Type of culture	**Yield (mol H_2 mol^{-1} glucose)**	**Yield (mLH_2 g^{-1}starch)**	**SHPR (mLH_2 g^{-1} cells h^{-1})**
ANS	1.14	222.85	32.10
MIX	0.87	133.09	27.76
CAB	0.83	125.53	34.22
CB	0.78	118.98	8.19
EA	1.04	159.04	10.40

Figure 5.2 depicts time course of variation of cumulative hydrogen, starch and glucose concentrations for the anaerobic sludge (ANS). Starch concentration decreased from nearly 6.8 gL^{-1} to 0.42 gL^{-1} after 330 h of fermentation indicating effective starch hydrolysis by ANS. Cumulative hydrogen increased rather fast within the first 150 h and reached nearly 560 mL (30 ^{o}C, 1 atm) at the end of 330 h. Sugar was an intermediary product in starch fermentation to VFA and hydrogen gas. Therefore, total sugar concentration increased from an initial level of 0.9 g L^{-1} to nearly 5 gL^{-1} within the first 40 h and then decreased to less than 1 gL^{-1} due to conversion to VFA and hydrogen which remained almost constant at 0.3 gL^{-1} for the rest of the fermentation period. The TVFA concentration increased steadily from 0.8 gL^{-1} to 2.8 gL^{-1} at the end of 330 hours.

Figure 5.3 depicts variation of total sugar concentrations with time for different anaerobic of cultures. The trends are in agreemente with dark fermentation scheme where starch is first hydrolyzed to sugars which was used by the bacteria to produce TVFA, hydrogen and CO_2. Sugar concentrations increased up to 4900 mgL^{-1} and then decreased below 1000 mgL^{-1} as a general trend. Total sugar concentrations were almost constant after 150 h due to effective fermentation of sugar to VFA and H_2.

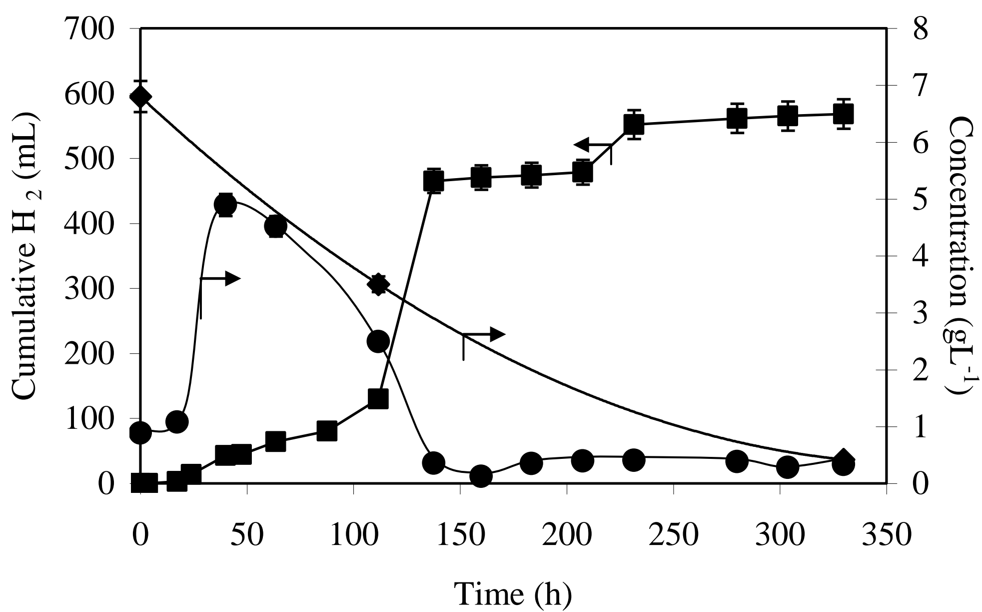


Figure 5.2 Variation of cumulative hydrogen (■), starch (♦) and total sugar (●) concentrations with time for the anaerobic sludge (ANS). Initial starch: 6800 mgL^{-1}.

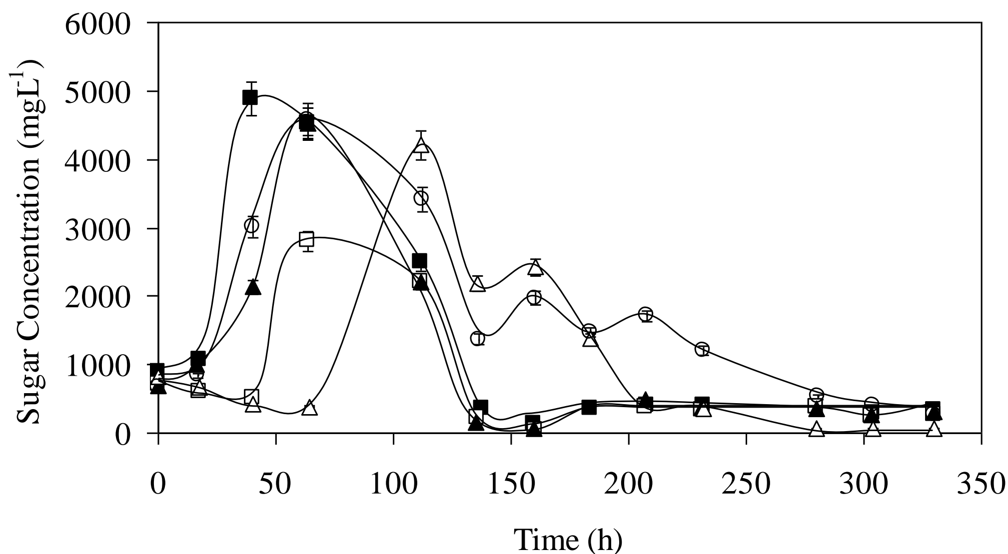


Figure 5.3 Variation of total sugar concentrations with time for different anaerobic cultures (■) ANS, (□) MIX, (▲) CAB, (Δ) CB, (○) EA.

The rates and yields of hydrogen formation varied depending on the type of cultures used due to differences in metabolic capabilities of the organisms. The highest hydrogen yield was obtained with the anaerobic sludge (ANS) followed by EA, MIX, CAB and CB cultures. Contribution of different spore forming hydrogen producers in ANS probably resulted in a more effective fermentation performance compared to other pure cultures. Anaerobic sludges from different sources had different hydrogen formation performances. The rate and the yield of hydrogen formation can be improved by addition of external nutrients to the fermentation media. Among the pure cultures, EA performed better than the CAB and CB, but was slower as compared to the ANS culture. Utilization of heat-treated anaerobic sludge (ANS) for bio-hydrogen production from waste ground wheat seems to be more suitable for long term operations without sterilization requirements.

Table 5.4 Comparison of hydrogen formation by dark fermentation of starch containing substrates using different anaerobic cultures.

Culture	**Operation mode**	**Substrate**	**Substrate concentration (gL^{-1})**	**H_2 Yield**	**Reference**
Clostridium butyricum	Batch	Pure starch	5	1.9 mol H_2 mol^{-1} glucose = 284 mL H_2 g^{-1} starch	Yokoi et al. (1998)
Clostridium butyricum + *Enterobacter aerogenes*	Batch	Sweet potatoe starch	10	2.4 mol H_2 mol^{-1} glucose = 357.5 mL H_2 g^{-1} starch	Yokoi et al. (2001)
Sewage sludge	Batch	Cassava starch	24 [a]	235 mLH_2 g^{-1}starch	Lee et al. (2008)
Sewage sludge	Continuous	Corn starch	20	0.92 mol H_2 mol^{-1} glucose =138 mL H_2 g^{-1} starch	Han et al. (2004)
Sewage sludge	Continuous	Wheat starch	10	64 mLH_2 g^{-1}starch	Asada et al. (2006)
Anaerobic sludge	Batch	Wheat starch	10	223mLH_2.g^{-1}starch= 1.14 mol H_2.mol^{-1} glucose	Our result

The results of this study are compared with other dark fermentation studies used for bio-hydrogen production from starch containing substrates in Table 5.4. Most of the reported studies used pure starch rather than powdered grain such as wheat, corn and rice. Hydrogen yield obtained with heat-treated anaerobic sludge (ANS) in this study (223 mLH_2 g^{-1}starch) is higher than some of the reported studies (Arooj et al.,

2008; Hussy et al., 2007). Slightly higher hydrogen yields were obtained in some reported studies Lee et al. (2008), Yokoi et al. (1998), Yokoi et al. (2001), due to addition of external nutrients to the fermentation medium.

5.1.1.2 Selection of Sludge Pre-treatment Method

A series of batch dark fermentation experiments were conducted for production of hydrogen from WPS solution. Anaerobic sludges from two different anaerobic treatment plants and their mixtures were subjected to repeated heat, chloroform and heat-chloroform combination treatments for selection of the most effective pre-treatment method.

Variations of cumulative hydrogen formation (CHF) with time for different pre-treatment methods and anaerobic sludges (ANS) are depicted in Figure 5.4. The highest CHF (652 mL) was obtained from the 10 h (2x 5h) boiled anaerobic sludge of PAK. Granular ANS of EFES and the mixture (MIX) of the two sludges yielded 211 mL and 350 mL H_2, respectively after 10 h (2x 5h) boiling. Apparently, the suspended anaerobic sludge (PAK) was more susceptible to heat treatment than the granular sludge. Heat pre-treatment was more effective than chemical treatment by chloroform alone since the lowest cumulative hydrogen was produced by the 0.05% chloroform treated mixed sludges (MIX) without heat treatment. CHF did not change significantly even with 0.05% and 0.1% chloroform treatments. The mixed sludge (MIX) exposed to 0.05% chloroform for 17 h before 5 h boiling showed a faster and more effective hydrogen production trend as compared to 0.1% chloroform treatment. Therefore, increases in chloroform concentration did not improve the hydrogen production significantly. As depicted in Figure 5.4, 0.05% chloroform addition to 10 h boiled MIX sludge increased the lag time as compared to only heat treated (10 h) MIX sludge. However, the amount of CHF reached almost the same level after about 135h fermentation indicating the fact that chloroform addition to 10 h boiled ANS had a negLigible effect on hydrogen production.

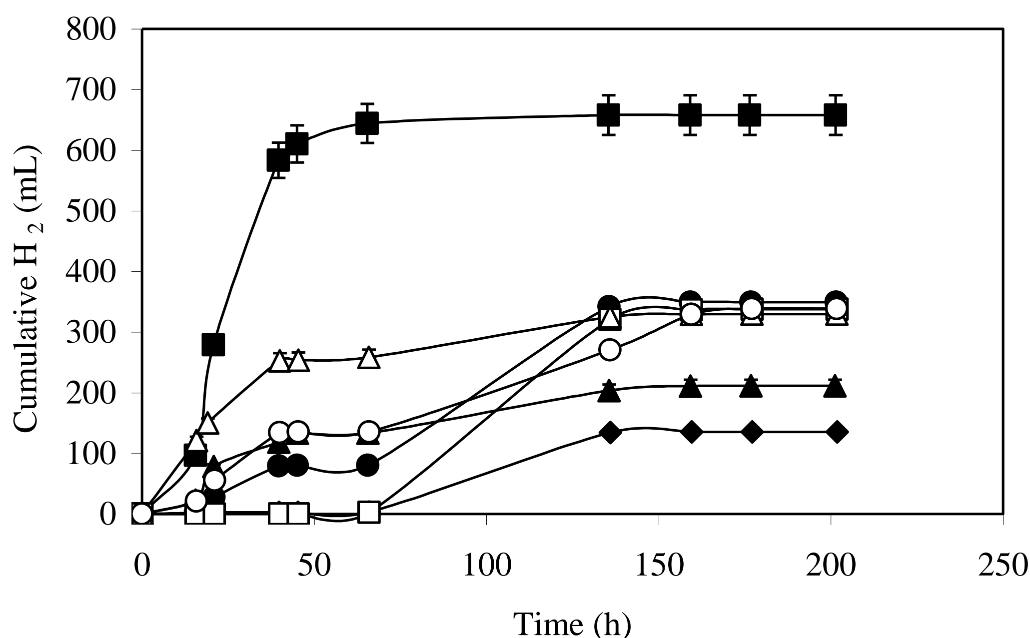


Figure 5.4 Variation of cumulative hydrogen with time for different pre-treatment methods (■) PAK (10h boiling), (▲) EFES (10h boiling), (●) MIX(10h boiling), (♦) MIX (only 0.05% chloroform treatment), (□) MIX (0.05 chloroform treatment after 10h boiling), (Δ) MIX (5h boiling after 0.05% chloroform treatment), (○) MIX (5h boiling after 0.1% chloroform treatment).

Cumulative hydrogen data depicted in Figure 5.4 were correlated with the Gompertz equation and the constants were determined by regression analysis as explained in section 0. Table 5.5 presents the Gompertz equation coefficients for different pre-treatment methods applied to different anaerobic sludges. The highest cumulative hydrogen formation (652 mL) was observed by 10 h (2x 5h) boiled PAK ANS with a R_m value of 17.4 mL h^{-1}. CHF of the MIX sludge (385 mL) was higher than the granular EFES sludge (209 mL) due to the presence of PAK sludge in the mixture. The MIX and the granular EFES sludges produced hydrogen with a rate of 3.73 mL h^{-1} and 2.27 mL h^{-1}, respectively after 10 h of heat pre-treatment alone. The lowest hydrogen formation (135 mL) was obtained with the MIX sludge pre-treated only with 0.05% chloroform with a hydrogen formation rate of R_m = 1.87 mL h^{-1} indicating ineffectiveness of chloroform pre-treatment alone. Chloroform treatment after 10h heat treatment did not improve hydrogen formation performance as

compared to 10 h heat treatment alone. Chloroform treatment before or after heat treatment was not as effective as the heat tratment alone. However, the rate of hydrogen formation (R_m) increased by chloroform treatment followed by 5h heat treatment as compared to only heat treatment to the MIX sludge. Hydrogen formation rate (R_m) increased from 3.73 mL h^{-1} to 6.31 mL h^{-1} when 5 h heat treatment was applied to the MIX culture after 0.05% chloroform treatment.

Table 5.5 Gompertz equation coefficients for different pre-treatment methods of various anaerobic sludges.

Type of Culture	Pre-treatment Method	P (mL)	R_m (mL.h^{-1})	λ (h)	R^2
PAKMAYA	10h (2x 5h) boiling	652.63	17.40	11.78	0.99
EFES	10h (2x 5h) boiling	208.91	2.27	20.83	0.96
MIXTURE	10h (2x 5h) boiling	385.09	3.73	15.5	0.97
MIXTURE	only 0.05% chloroform treatment	135.55	1.87	69.85	0.99
MIXTURE	0.05% chloroform treatment after 10h boiling	338.90	4.55	74.91	0.99
MIXTURE	5h boiling after 0.05% chloroform treatment	328.89	6.31	1.13	0.97
MIXTURE	5h boiling after 0.1% chloroform treatment	362.27	2.14	1.58	0.97

Table 5.6 summarizes hydrogen yields and SHPR for different pre-treatment methods and anaerobic sludges. The highest yield was obtained by 10 h boiled PAK sludge (1 mol H_2 mol^{-1} glucose = 150.8 mL H_2 g^{-1}) followed by 10 h boiled MIX sludge (0.52 mol H_2 mol^{-1} glucose). Chloroform (0.05%) treatment of the MIX sludge after 10 h boiling resulted in 0.51 mol H_2 mol^{-1} glucose. Almost the same hydrogen yields were obtained for the combination of 5 h boiling after 0.05% (0.44 mol H_2.mol^{-1} glucose) or 0.1% (0.47 mol H_2 mol^{-1} glucose) chloroform treatment. Chloroform pre-treatment alone applied to the MIX sludge gave the lowest yield of 0.19 mol H_2 mol^{-1} glucose. Yield values in terms of mLH_2.g^{-1}starch are also presented in Table 5.6 with the highest value (150.8 mL H_2 g^{-1} starch) obtained with 10 h boiled PAK sludge and the lowest value (29.6 mL H_2 g^{-1} starch) with the chloroform treated MIX sludge. The SHPR values are in agreement with the R_m values presented in Table 5.5 since SHPR was calculated using SHPR = $R_m/(V_o\ X_o)$ where the dominator was constant for every culture. The highest SHPR was also obtained with the 10 h boiled PAK sludge (25.7 mL H_2 g^{-1} biomass h^{-1}) and the

lowest was with the chloroform treatment alone (2.76 mL H_2 g^{-1} biomass h^{-1}). Chloroform treatment alone is not recommended for selection of the hydrogen producing bacteria.

Table 5.6 Yields and specific hydrogen production rates (SHPR) for different pre-treatment methods of various anaerobic sludges.

Type of Culture	**Pre-treatment Method**	**Yield ($molH_2mol^{-1}glucose$)**	**Yield ($mLH_2g^{-1}starch$)**	**SHPR ($mLH_2g^{-1}cellsh^{-1}$)**
PAKMAYA	10h boiling	1.00	150.85	25.71
EFES	10h boiling	0.33	50.84	3.35
MIXTURE	10h boiling	0.52	79.52	5.51
MIXTURE	only 0.05% chloroform treatment	0.19	29.62	2.76
MIXTURE	0.05% chloroform treatment after 10h boiling	0.51	77.11	6.72
MIXTURE	5h boiling after 0.05% chloroform treatment	0.44	67.46	9.32
MIXTURE	5h boling after 0.1% Chloroform treatment	0.47	71.70	3.16

Table 5.7 presents a comparison of some reported pre-treatment methods applied to different anaerobic sludges used for hydrogen production by dark fermentation of starch containing substrates. Application of heat pre-treatment to anaerobic sludges with various retention times was always effective in selecting hydrogen producing bacteria. Short heat treatment periods (15 min) yielded relatively low hydrogen yields (Arooj et al., 2008; Hawkes et al., 2007). The highest hydrogen yield was reported to be 1.55 $moles^{-1}$ H_2 mol^{-1} glucose when municipal sewage sludge was heat treated for 1h (Lee et al., 2008). Hydrogen producers can also be selected by operating a chemostat at low hydraulic residence times. Yang et al. (2006) selected the hydrogen producers by holding the hydraulic retention time at 12 hours in a continuous fermenter operation. Hydrogen consumers were probably washed out at low HRTs since their growth rates were lower than the hydrogen producers. Hydrogen yields vary with the composition of the bacterial culture along with the other parameters such as the composition of the nutrient media, environmental

conditions and also the type of the substrate. All other parameters were kept constant in this study, except the pre-treatment method and the source of anaerobic sludge was different. The hydrogen yields obtained in this study are comparable with the literature studies (Arooj et al., 2008; Hawkes et al., 2008; Lee et al., 2008). The PAK anaerobic sludge with 10 h heat pre-treatment yielded the highest hydrogen formation. Chloroform treatment should not be used alone, but with the heat treatment, if necessary.

Table 5.7 Comparison of different pre-treatment methods and the sludges used for dark fermentative hydrogen gas production from various starch sources.

Seed	Pretreatment method	Substrate	H_2 Yield	Reference
Anaerobic digester sludge	Heated at 110 °C for 15 min	25gL^{-1} Wheat hydrolysate	0.9 mol H_2.mol^{-1}hexose	Hawkes et al. (2007)
Anaerobic digester sludge	Heated at 90 °C for 10 min	20gCODl^{-1} corn starch	0.92 mol H_2.mol^{-1} glucose	Arooj et al. (2008)
Municipal sewage sludge	Heated at 95-100°C for 1h	24gCODl^{-1} Cassava starch	9.47mmolH_2.g^{-1}starch=1.55molH2.mol^{-1}glucose	Lee et al. (2008)
Mixed microflora	No heat treatment, HRT=12h	20gL^{-1} soluble starch	296.2 mLH_2.g^{-1}starch=2.16molH_2.mol^{-1}glucose	Yang et al. (2006)
Anaerobic sludge	5h repeated boiling	20gL^{-1} WPS	1 mol H_2 mol^{-1} glucose	Our Result

5.1.1.3 Effects of Wheat Powder Boiling on Hydrogen Production

The effects of boiling of WPS on dark fermentative hydrogen gas production was investigated in two series of batch experiments. Figure 5.5 illustrates variations of cumulative hydrogen gas production for the boiled and unboiled WPS. Cumulative hydrogen increased until 25h of fermentation period for both cases and then remained stable. Boiled and unboiled WPS resulted in 446 mL and 433 mL cumulative hydrogen gas at the end of experiments. However, fermentation occurred faster when WPS was boiled.

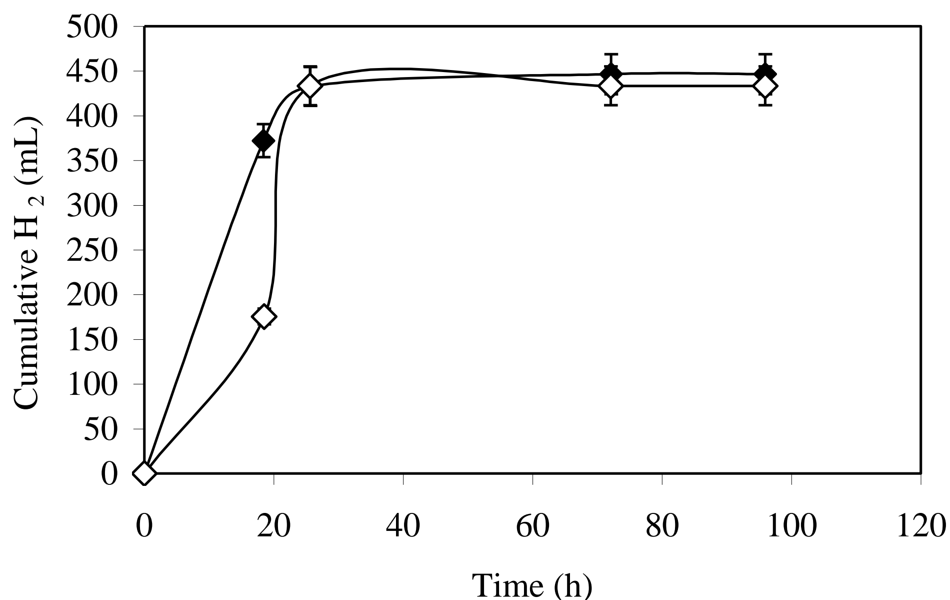


Figure 5.5 Variation of cumulative hydrogen gas volume with time for boiled and unboiled WPS in dark fermentation. (♦) Boiled WPS, (◊) Unboiled WPS.

Figure 5.6 presents the variation of cumulative hydrogen, starch, TVFA and glucose concentrations with time for boiled WPS. Active fermentation took place within 25 hours since cumulative hydrogen volume reached its maximum value and did not change until the end of fermentation period. Starch was effectively converted to TVFA. The initial TVFA concentration increased from 0.39 gL^{-1} to 4.8gL^{-1} and starch concentration decreased from 7.9 gL^{-1} to 2.3 gL^{-1} respectively. Starch conversion to TVFA was about %60. Glucose was also consumed in parallel with starch consumption. Final starch and glucose concentrations were 1 gL^{-1} and 0.57 gL^{-1}, respectively.

Figure 5.7 presents the variation of cumulative hydrogen formation, starch, glucose and TVFA concentrations with time for the dark fermentation of unboiled WPS with ANS. 433 mL cumulative hydrogen was produced within 25 hours and then remained stable. Starch concentration decreased from 8.13 gL^{-1} to 1.5gL^{-1} in 25h then reached 0.736 gL^{-1} at the end of fermentation. Since the glucose was simultaneously consumed with starch, no increase in glucose concentration was observed.

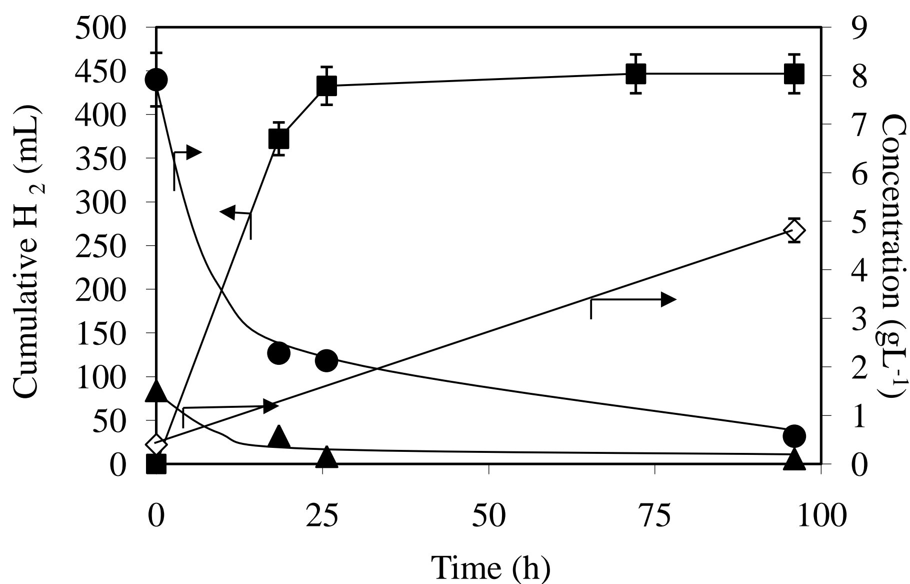


Figure 5.6 Variation of (■) cumulative hydrogen (mL) , (●) starch (gL^{-1}), (◊) TVFA (gL^{-1}) and (▲) glucose (gL^{-1}) concentrations with time for boiled WPS.

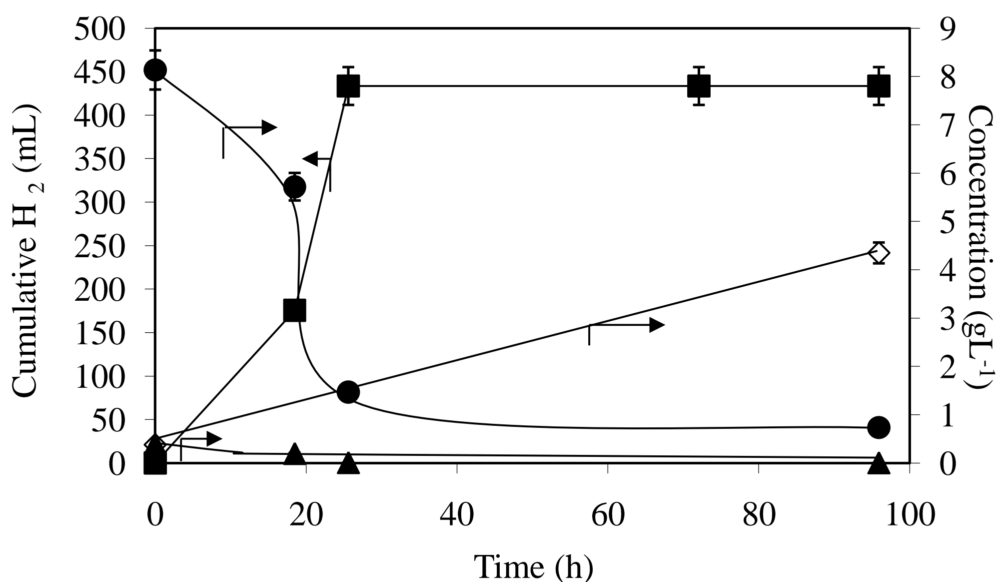


Figure 5.7 Variation of (■) cumulative hydrogen (mL) , (●) starch (gL^{-1}), (◊) TVFA (gL^{-1}) and (▲) glucose (gL^{-1}) concentrations with time for unboiled WPS.

Data obtained from boiled and unboiled WPS in Figure 5.5 were correlated with Gompertz equation and the constants were determined as explained in section 4.1.2. As presented in Table 5.8 the hydrogen production potential for both series of experiments were almost the same. But a distinct difference was observed for the rates which were R_m= 20 mL h^{-1} for boiled WPS and R_m= 16.90 mL h^{-1} for unboiled WPS. The lag times for boiled and unboiled WPS were 7 h and 17h respectively. This may be a result of the presence of readily available glucose in boiled WPS which was produced with the partial hydrolysis of WPS during boiling. In unboiled WPS, the bacteria had to hydrolyse starch to glucose which resulted in a delay in hydrogen formation.

Table 5.8 Gompertz equation constants for dark fermentative hydrogen production from boiled and unboiled WPS.

Pre-treatment	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
Boiled WPS	446.45	20.00	7.39	1.00
Unboiled WPS	433.52	16.90	17.17	0.99

Effects of boiling on the specific hydrogen production rate (SHPR) and hydrogen formation yield are presented in Table 5.9. Altough same yields were obtained for boiled and unboiled WPS, the rates were different. The SHPR for boiled and unboiled WPS were 55.17 mL H_2 g^{-1} biomass h^{-1} and 46.62 mL H_2 g^{-1} biomass h^{-1}, respectively.

Partial hydrolysis of wheat starch by boiling prior to dark fermentation significantly increased the SHPR and decreased the lag period. Therefore, it was decided to used boiled and partially hydrolyzed wheat starch in further experiments.

Table 5.9 Effects of partial hydrolysis of WPS by boiling on hydrogen formation yield and SHPR in dark fermentation.

Pre-treatment	**$Starch_o$ (mgL^{-1})**	**$Starch_F$ (mgL^{-1})**	**Yield (mL H_2 g^{-1} starch)**	**Yield ($molH_2$ mol^{-1} glucose)**	**SHPR (mLH_2 g^{-1} biomass h^{-1})**
Boiled WPS	7917.77	572.70	209.90	1.40	55.17
Unboiled WPS	8134.36	736.14	202.10	1.35	46.62

5.1.1.4 Effects of C/N and C/P Ratios

Variations of hydrogen yield and formation rate with the C/N and C/P ratio were investigated by using a Box-Wilson statistical experiment design approach and the C/N and C/P ratios yielding the highest yield and the rate were determined. The experimental conditions of the Box- Wilson statistical design are presented in Table 4.1. The results of the Box-Wilson design experiments are presented in Table 5.10 along with the predicted values from the response functions.

Table 5.10 Experimental and predicted values of the yields and SHPR

Experiment	**Yield$_{(exp)}$ (mgH_2 g^{-1} starch)**	**Yield$_{(pred)}$ (mgH_2 g^{-1} starch)**	**SHPR$_{(exp)}$ (mgH_2 g^{-1} bm h^{-1})**	**SHPR$_{(pred)}$ (mgH_2 g^{-1} bm h^{-1})**
A1	7.82	8.73	2.27	2.46
A2	14.25	14.89	5.53	5.20
A3	9.41	8.46	3.19	2.16
A4	6.42	8.92	1.77	2.67
F1	17.87	15.96	5.62	5.19
F2	8.37	8.89	2.06	2.99
F3	6.95	4.85	2.21	1.41
F4	10.94	11.28	2.32	2.89
C (ave)	6.74	6.74	1.84	1.84
No N-P	6.09	-	2.36	-

The coefficients of the response functions determined by regression analysis are summarized in Table 5.11 and were used to predict the response function values presented in Table 5.10.

Table 5.11 Coefficients of the response functions for different C/N and C/P ratios

	b_0	**b_1**	**b_2**	**b_{12}**	**b_{11}**	**b_{22}**	**R^2**
Yield	21.806	-0.186	-0.026	1.58×10^{-4}	6.26×10^{-4}	9.0×10^{-6}	0.86
SHPR	6.034	-0.061	-0.006	4.30×10^{-5}	2.45×10^{-4}	2.5×10^{-6}	0.78

Hydrogen yield predictions were in good agreement with the experimental results (R^2 = 0.86) as shown in Table 5.10. A control experiment was conducted without any N and P addition. The results of this experiment are also presented in Table 5.10 (No N-P addition).

Figure 5.8 depicts variation of cumulative hydrogen (mL), starch, glucose and TVFA concentrations (mg L^{-1}) with time for the experiment F1 where C/N and C/P

ratios were 173 and 860, respectively since the hydrogen yield and the rate were the highest for this experiment. Starch concentration decreased from an initial value of nearly18 gL^{-1} to 2 gL^{-1} after 150 hours of fermentation indicating effective starch hydrolysis by the anaerobic sludge bacteria. Cumulative hydrogen volume increased rather fast within the first 100 hours of fermentation and reached nearly 3650 mL (30 ^{o}C, 1 atm) at the end of 200 hours of incubation which is equivalent to 3590 mL at STP (25 ^{o}C, 1atm). Sugar was an intermediary product in starch fermentation to organic acids and hydrogen gas. Therefore, total sugar concentration increased from an initial level of 2 gL^{-1} to nearly 9 gL^{-1} within 50 hours and then decreased to less than 1 gL^{-1} due to sugar conversion to VFA and hydrogen gas. Sugar concentration remained almost constant at 0.4 gL^{-1} for the rest of the fermentation period. The TVFA concentration increased steadily from 2 gL^{-1} to 11 gL^{-1} at the end of 300 hours of fermentation. HPLC analysis of VFA indicated that the major organic acids were acetic, butyric, propionic and lactic acids.

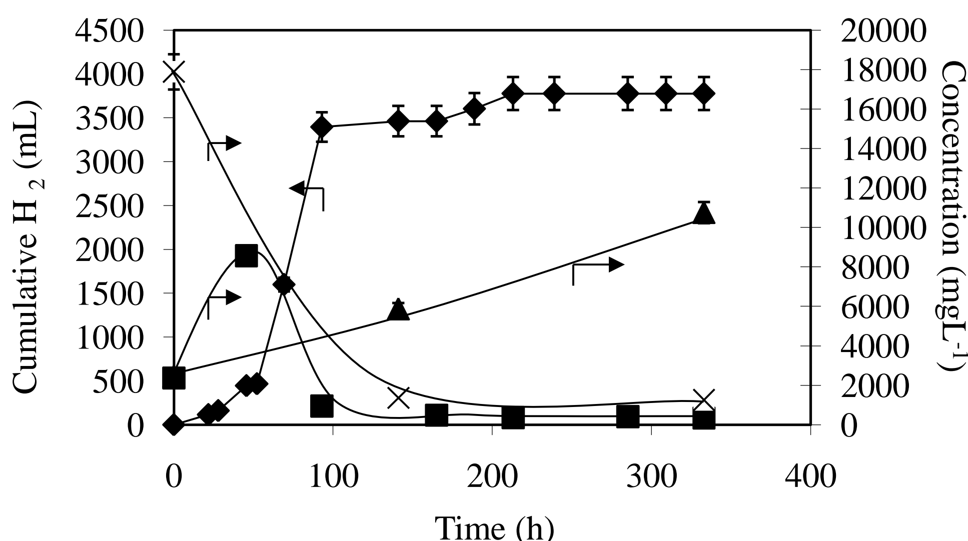


Figure 5.8 Variation of cumulative hydrogen (♦), starch (X), TVFA (▲), and total sugar (■) concentrations with time for the experiment F1 (C/N = 173, C/P = 860).

The response functions with determined coefficients were used to predict the variations of hydrogen yield and formation rate with the C/N and C/P ratio.

Variations of hydrogen yield (mg H_2 g^{-1}starch) with C/P ratio at different C/N ratios are depicted in Figure 5.9.

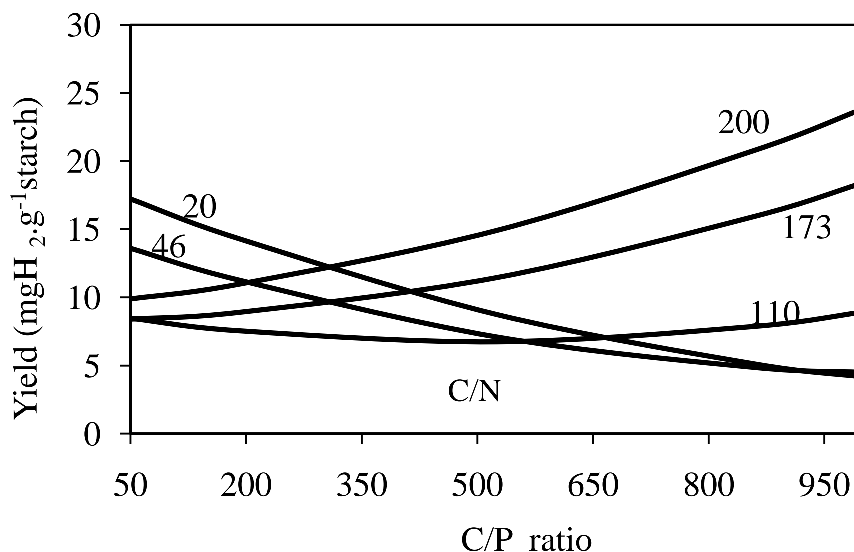


Figure 5.9 Variation of hydrogen yield with the C/P ratio at different C/N ratios.

At low C/N ratios (< 46) indicating excess nitrogen (high N/C ratio), hydrogen yield increased with decreasing C/P or increasing P/C ratio indicating high phosphorous requirements at high nitrogen concentrations. At high C/N ratios (>110) indicating Low nitrogen contents (low N/C ratios), hydrogen yield increased with increasing C/P or decreasing P/C ratio indicating Low phosphorous requirements at low nitrogen contents. At a high C/P ratio of 950 (low P content) hydrogen yield increased with increasing C/N or decreasing N/C ratio indicating Low nitrogen requirements at low phosphorous contents. N and P requirements were proportional with each other and also with the carbon or starch content of the wheat due to prevailing stoichiometry of the anaerobic metabolism. The highest hydrogen yield (23 mg H_2 g^{-1}starch or 281 mL H_2 g^{-1}starch at STP) was obtained at the highest C/N and C/P ratios of 200 and 1000 corresponding the lowest N/C and P/C ratios of 0.005 and 0.001. The C/N/P ratio maximizing hydrogen yield was 100/0.5/0.1 corresponding to a starch/ N/ P ratio of 100/ 0.2/ 0.04 (w $w^{-1}w^{-1}$). The results indicated that the external nitrogen and phosphorous requirements of the anaerobic sludge organisms for the highest hydrogen yield was 0.2% and 0.04% of dry weight

starch or 0.5% and 0.1% of total organic carbon available. Low external N and P requirements are partly due to presence of N and P in the WP and partly due to low growth yields of the anaerobic organisms (0.06 g biomass g^{-1}sugar).

Figure 5.10 depicts variation of hydrogen yield (mg H_2 g^{-1}starch) with C/N ratio at different C/P ratios. At low C/P ratios (< 525, excess P), hydrogen yield decreased with increasing C/N ratio (decreasing N/C ratio) indicating Limitations by the nitrogen source at high phosphorous contents. However, at high C/P ratios (> 525) or low P contents hydrogen yield increased with increasing C/N ratio (decreasing N/C ratio) steadily indicating Low nitrogen requirements at low phosphorous concentrations.

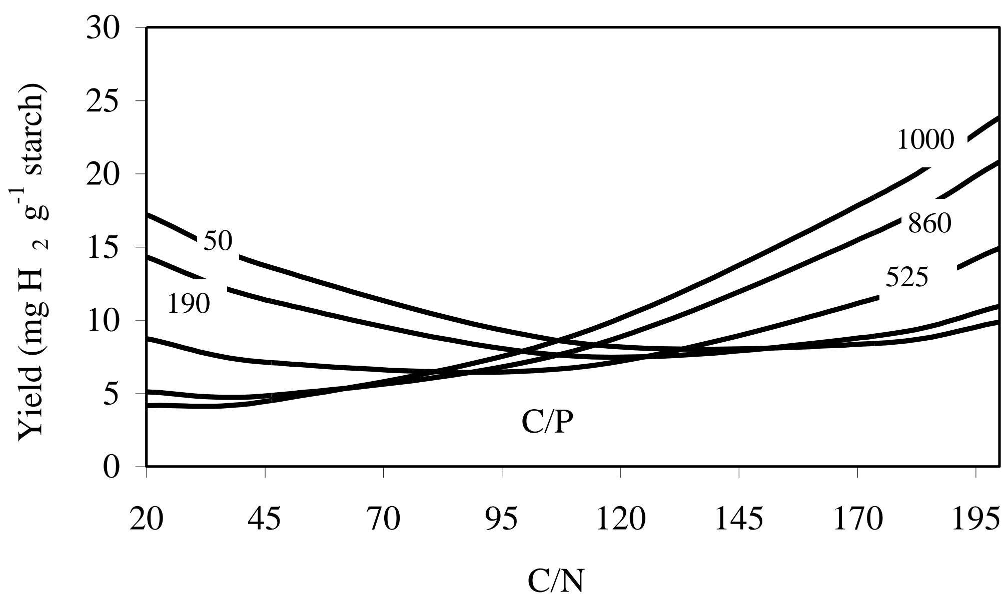


Figure 5.10 Variation of hydrogen yield with the C/N ratio at different C/P ratios.

Similarly, at a low C/N ratio of 45 indicating presence of excess nitrogen, hydrogen yield increased with decreasing C/P ratio or increasing P/C ratio indicating the requirement for high phosphorous contents. At high C/N ratios of 195 and above indicating Low nitrogen content, hydrogen yield increased with increasing C/P or decreasing P/C ratio indicating Low P requirements. For a constant carbon content of the medium (e.g., 20 g starch l^{-1} or 8 g C l^{-1}) the nitrogen and phosphorous contents should change in the same direction requiring Low P for low N contents for high

hydrogen yield due to prevailing stoichiometry of anaerobic metabolism. Within the range of independent variables, the highest hydrogen yield (23 mg H_2 g^{-1} starch or 281 mL H_2 g^{-1}starch at STP) was obtained with the highest C/N and C/P ratios of 200 and 1000 or the lowest N/C and P/C ratios of 0.005 and 0.001. N/ P ratio maximizing the hydrogen yield was 5. The hydrogen yield obtained with no nitrogen and phosphorous addition was 6.09 mg H_2 g^{-1}starch or 74.4 mL H_2 g^{-1} starch at STP which was considerably lower than the maximum yield obtained at C/N = 200 and C/P =1000. The N and P content of the WP was not sufficient and external addition of N and P was required for effective hydrogen production by dark fermentation of WPS.

The specific hydrogen production rate (SHPR) is another important parameter indicating effectiveness of the fermentation. SHPR was calculated as the mg or mL hydrogen produced per gram dry weight of initial biomass per unit time (mg H_2 g^{-1}biomass h^{-1}) at the end of 240 hours where cumulative hydrogen production was leveled off. The following equation was used in SHPR calculations,

$$\text{SHPR} = \frac{\text{mg or mL } H_2 \text{ produced}}{X_{To}.t} \qquad \text{Eqn5.1}$$

where mg or mL H_2 produced is the amount of cumulative hydrogen produced in 240 hours, X_{To} is the initial amount of biomass (g, X_o V_o) and t is the fermentation period (240 h).

Variation of SHPR with C/P ratio at different C/N ratios is depicted in Figure 5.11. At low C/N ratios (< 46), or high nitrogen contents, the SHPR decreased with increasing C/P ratio due to limitations caused by low phosphorous content when nitrogen is in excess. However, at high C/N ratios (> 46) where nitrogen was the rate limiting substrate, the SHPR increased with increasing C/P ratio indicating Low phosphorous requirements when nitrogen concentration was low. N and P requirements were proportional as dictated by the stoichiometry of anaerobic metabolism. The highest SHPR (8.0 mg H_2 g^{-1}biomass.h^{-1} or 98 mL H_2 g^{-1}biomass h^{-1} at STP) was obtained at C/N = 200 and C/P =1000 indicating Low nitrogen and

phosphorous requirements for maximum rate of hydrogen formation. The C/N/P ratio yielding the highest SHPR was 100/0.5/0.1. The SHPR obtained with no nitrogen and phosphorous addition was 2.36 mg H_2 g^{-1}biomass h^{-1} or 28.8 mL H_2 g^{-1}biomass h^{-1} which was considerably lower than that of the maximum SHPR indicating the need for external nitrogen and phosphorous addition to yield C/N/P = 100/0.5/0.1 in the fermentation medium.

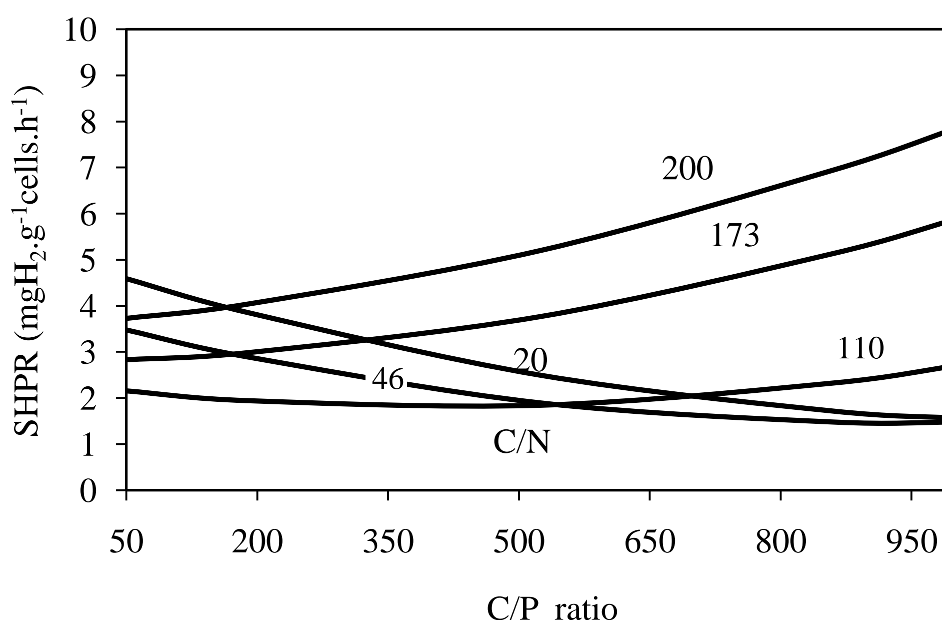


Figure 5.11 Variation of SHPR with the C/P ratio at different C/N ratios.

Figure 5.12 depicts variation of SHPR with the C/N ratio at different C/P ratios. At low C/P ratios (< 525, high P content), SHPR decreased with increasing C/N ratio up to C/N = 95 indicating Limitations by the available nitrogen when phosphorous was in excess. However, at high C/N ratios above 95, the SHPR increased steadily with increasing C/N ratio indicating Low requirement for nitrogen source. Similarly, at high C/N ratios above 95 (low nitrogen content), the SHPR increased with increasing C/P ratio due to low phosphorous requirements by the organisms. The highest SHPR (8.0 mg H_2 g^{-1}biomass h^{-1} or 98 mL H_2 g^{-1}biomass h^{-1} at STP) was obtained at the highest C/N and C/P ratios of 200 and 1000, respectively. The optimal C/N/P ratio maximizing the SHPR was found to be 100/0.5/0.1. Low

nitrogen and phosphorous requirements are due to low growth yields in anaerobic organisms.

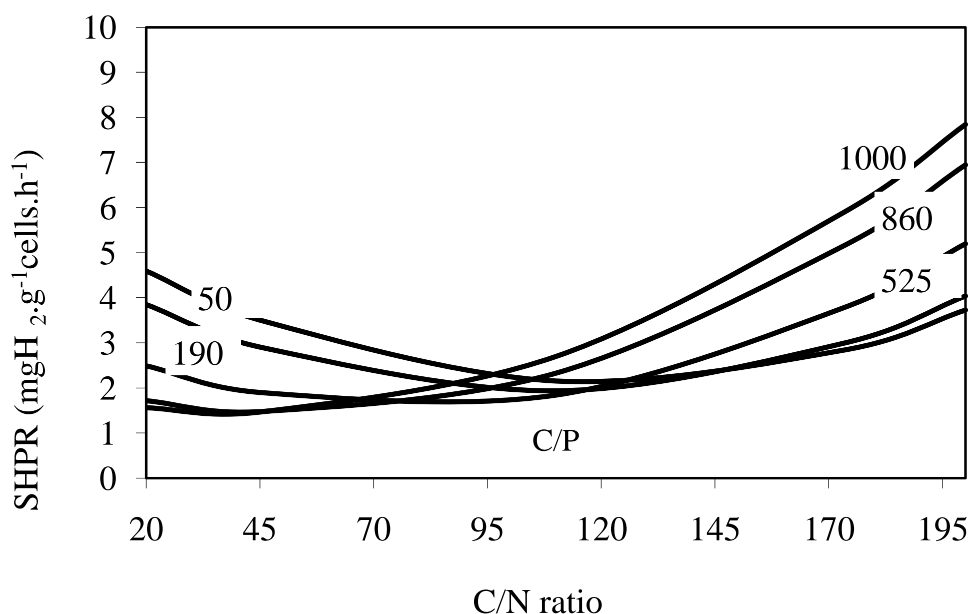


Figure 5.12 Variation of SHPR with the C/N ratio at different C/P ratios.

The highest hydrogen yield obtained in this study (281 mLH_2 g^{-1}starch at STP) corresponds to a hydrogen yield of nearly 2 $molesH_2$ $mole^{-1}$glucose which is comparable with the literature studies reporting hydrogen production from starch by dark fermentation (Yokoi et al., 1998; Yokoi et al., 2001). Liu & Shen (2004) studied hydrogen production from starch by dark fermentation and obtained a maximum hydrogen yield of 194 mL H_2 g^{-1} starch and hydrogen formation rate of 9.8 mL H_2 g^{-1}biomass h^{-1}at STP with a starch concentration of 15 gL^{-1}. Our results were considerably higher than those reported by Liu & Shen (2004) due to external addition of nitrogen and phosphorous and using the most suitable C/N and C/P ratios to meet nutritional requiremens of the organisms. In a study by Lin & Lay (2004) the optimal C/N ratio was reported 47 for biohydrogen formation from sucrose solution. Since part of the nitrogen was provided by the wheat in our study, the optimal C/N ratio was found to be 200. The external nitrogen requirement in our case was lower making the process economically more attractive.

Theoretical, hydrogen yields are 4 moles H_2 mole^{-1}glucose and 2 moles of H_2 mole^{-1} glucose when acetic (2 moles) or butyric (1 mole) acids were the only end products from one mole of glucose, respectivly. Formation of propionic acid (2 moles) consumes 2 moles of hydrogen per mole of glucose. That is propionic acid formation is a hydrogen consuming reaction and should be avoided. Lactic acid and ethanol formations do not yield hydrogen gas.

Since a mixture of acetic, butyric, propionic and lactic acids were produced in this study, the highest hydrogen yield was estimated to be 2 moles H_2 mole^{-1}sugar. At the experimental point of F1, 9072 mgL^{-1} TVFA was produced at the end of the fermentation which is nearly 50% of the initial starch. TVFA's consisted of acetic, butyric, lactic and propionic acids with the concentrations of 3755 mgL^{-1} (41%), 1627 mgL^{-1} (18%), 1130 mgL^{-1} (12.5%) and 1520 mgL^{-1} (17%), respectively.

5.1.1.5 Effects of Initial WP and Biomass Concentrations

Effects of initial WP (starch) and biomass concentrations on hydrogen production rate and yield in dark fermentation of WP solution by heat treated anaerobic-acidogenic sludge was investigated in batch experiments.

5.1.1.5.1 Effects of Initial Wheat Powder Concentration. Figure 5.13 depicts variation of cumulative hydrogen with time for different WP concentrations when the initial cell concentration was 2.6 gL^{-1}. Cumulative hydrogen increased with time and reached the maximum level after nearly 120 h (5 d). Cumulative hydrogen increased with increasing wheat powder concentration up to 20 gL^{-1} due to substrate limitations at low WP concentrations. Further increases in the WP concentration resulted in lower cumulative hydrogen formation probably due to substrate and product (VFA) inhibition. The highest cumulative hydrogen (751 mL) was obtained with a wheat powder concentration of 20 gL^{-1}. Apparently, wheat starch concentrations above 20 gL^{-1} caused substrate and product (high VFA formtion) inhibition yielding Low hydrogen formation. The optimum initial biomass/substrate ratio (Xo/So) was found to be 0.13 g biomass g^{-1} starch.

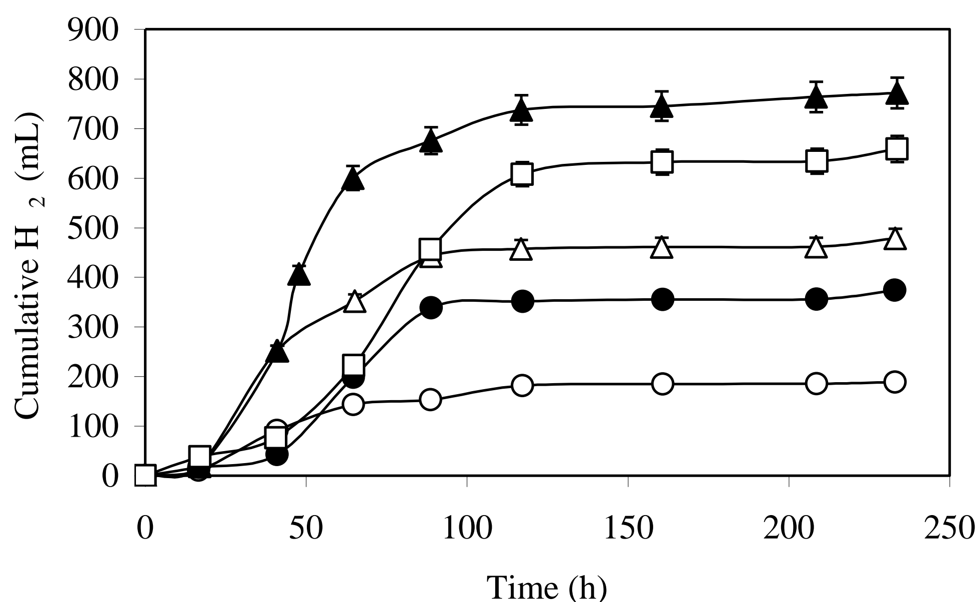


Figure 5.13 Variation of cumulative hydrogen gas with time for different wheat powder (starch) concentrations. o 5 g L^{-1}, ● 10 g L^{-1}, Δ 15g L^{-1}, ▲ 20g L^{-1}, □ 30 g L^{-1}

Cumulative hydrogen data depicted in Figure 5.13 were correlated with the Gompertz equation and the constants were determined by regression analysis according section 0. As presented in Table 5.12 the highest cumulative hydrogen (751 mL) and the formation rate (16.7 mL h^{-1}) were obtained with 20 gL^{-1} WP concentration. However, the lowest lag phase (13 h) was obtained with a WP concentration of 5 gL^{-1} due to high biomass/substrate ratio at low WP concentrations.

Table 5.12 Gompertz eqn. coefficients for different initial WP concentrations ($X_o = 2.6$ g L^{-1})

Initial WP concentration (g L^{-1})	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
5	184.01	3.02	13.05	0.99
10	363.49	8.16	38.58	0.99
15	464.38	8.81	14.88	0.99
20	751.26	16.71	24.65	0.99
30	652.60	9.80	39.57	0.99

The initial and the final concentrations of WP-starch and TVFA are summarized in Table 5.13 for different initial WP concentrations. Starch content of wheat powder was almost completely hydrolyzed for all cases. Final VFA concentrations increased

with increasing initial WP-starch concentration as expected. High TVFA concentrations produced at high initial WP-starch concentrations yielded low hydrogen formation due to substrate and product inhibition.

Table 5.13 Initial and final starch and TVFA concentrations for the experiments with varying initial WP concentration

$Starch_o$ (gL^{-1})	**$Starch_F$ (gL^{-1})**	**$TVFA_o$ (gL^{-1})**	**$TVFA_F$ (gL^{-1})**
5.03	0.32	0.39	2.74
10.30	0.58	0.59	4.57
14.34	0.89	0.67	5.50
20.91	1.26	0.48	7.33
29.50	2.21	0.65	11.70

Variations of hydrogen (mL H_2 g^{-1} starch) and VFA (mg VFA g^{-1}starch) yields with initial WP concentration are depicted in Figure 5.14. Hydrogen yield coefficient (mL H_2 g^{-1} starch) was almost constant at 96 ±2 mL H_2 g^{-1} starch (30 ^oC, 1 atm) for the initial WP (starch) contents between 5 and 20 gL^{-1}. Further increase in WP concentration to 30 gL^{-1} resulted in a decrease in hydrogen yield to 60 mL H_2 g^{-1} starch due to high concentrations of VFA causing product inhibition. The theoretical hydrogen yield from starch is 550 mL H_2 g^{-1} starch in dark fermentation if acetic acid is the only product. Our results are lower than that since a mixture of VFA's were produced. The produced hydrogen may have been consumed by the homo-acetogenic bacteria for VFA formation using the uptake hydrogenase enzyme. TVFA yields decreased slightly with increasing WP concentrations and reached a minimum level (348 mg TVFA g^{-1}starch) at WP = 20 gL^{-1} which further increased to 405 mg TVFA g^{-1} starch at the WP concentration of 30 gL^{-1}.

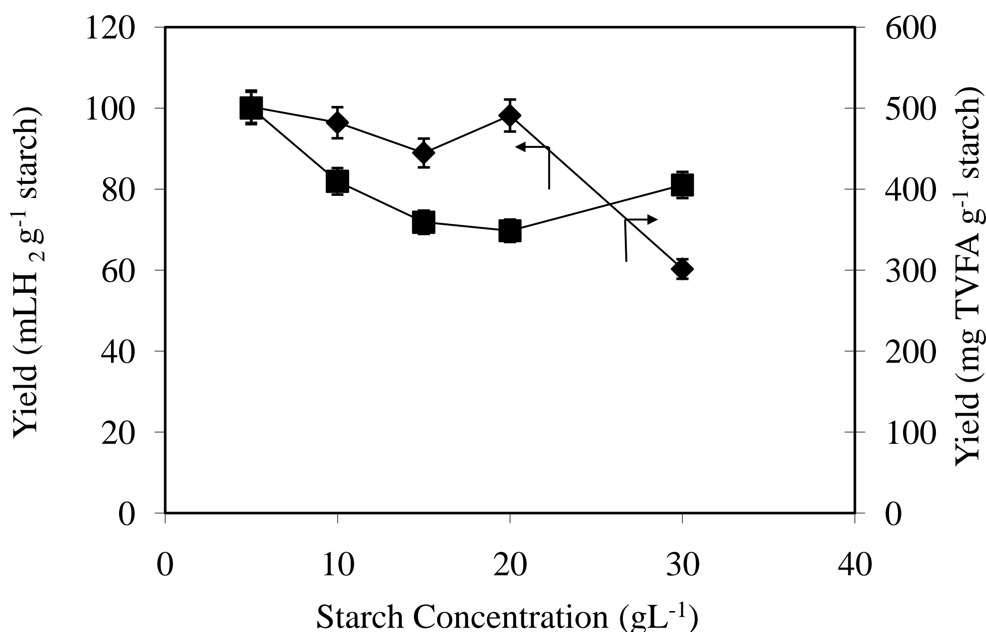


Figure 5.14 Variation of hydrogen (♦) and TVFA yields (■) for different wheat powder (starch) concentrations.

High hydrogen yields resulted in low TVFA yields due to changes in metabolic pathways depending on the composition of the microbial flora and the starch concentration. Apparently, high starch concentrations above 20 gL^{-1} shifted the bacterial metabolism towards VFA formation rather than hydrogen formation. Excess VFA formation at high initial starch concentrations may have inhibited hydrogen formation too (Table 5.13). In other words, H_2 / VFA ratio decreased for WP concentrations above 20 gL^{-1}. The optimum WP concentration resulting in the highest hydrogen and the lowest VFA yields was 20 gL^{-1} corresponding an optimum Xo/So ratio of 0.13 g biomass g^{-1} starch.

Variation of SHPR's with the WP concentration is depicted in Figure 5.15. SHPR increased with increasing initial WP concentration up to 20 g WP L^{-1} due to substrate limitations at low WP concentrations. WP concentrations above 20 gL^{-1} caused substrate and product inhibitions due to high starch and VFA concentrations. The optimum Xo/So ratio maximizing the SHPR (16.1 mL H_2 g^{-1} biomass h^{-1}) was 0.13 g biomass g^{-1} starch.

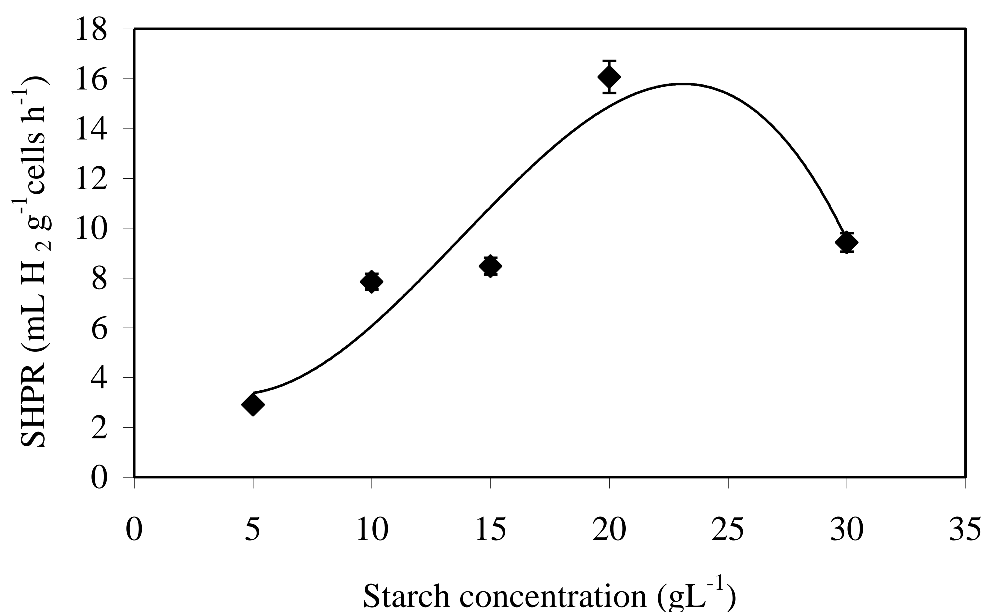


Figure 5.15 Variation of specific hydrogen production rate (SHPR) with the initial wheat powder (starch) concentrations.

5.1.1.5.2 Effects of Initial Biomass Concentration. Variations of cumulative hydrogen (mL) with time are depicted in Figure 5.16 for different initial biomass concentrations between 0.5 and 5 gL^{-1} while the initial WP was 20 gL^{-1}. Cumulative hydrogen increased with time and reached a constant level within 50 hours of incubation for all biomass concentrations except the one for 0.5 gL^{-1} where the lag phase was considerably high due to low biomass/ substrate ratio. Cumulative hydrogen formation increased with the initial biomass concentration up to 2.5 gL^{-1} due to limitations by the low biomass concentration. However, further increases in initial biomass concentration resulted in lower cumulative hydrogen formation probably due to consumption of hydrogen by the homo-acetogenic bacteria producing acetic acid from H_2 and CO_2 or activation of uptake hydrogenase enzyme. The results indicated an optimum inital biomass/substrate ratio of Xo/So = 0.125 g biomass g^{-1} WP yielding the highest cumulative hydrogen formation (175 mL H_2). This ratio is almost the same as the ratio determined from the variable substrate (WP) concentration experiments (0.130 g biomass g^{-1}WP) .

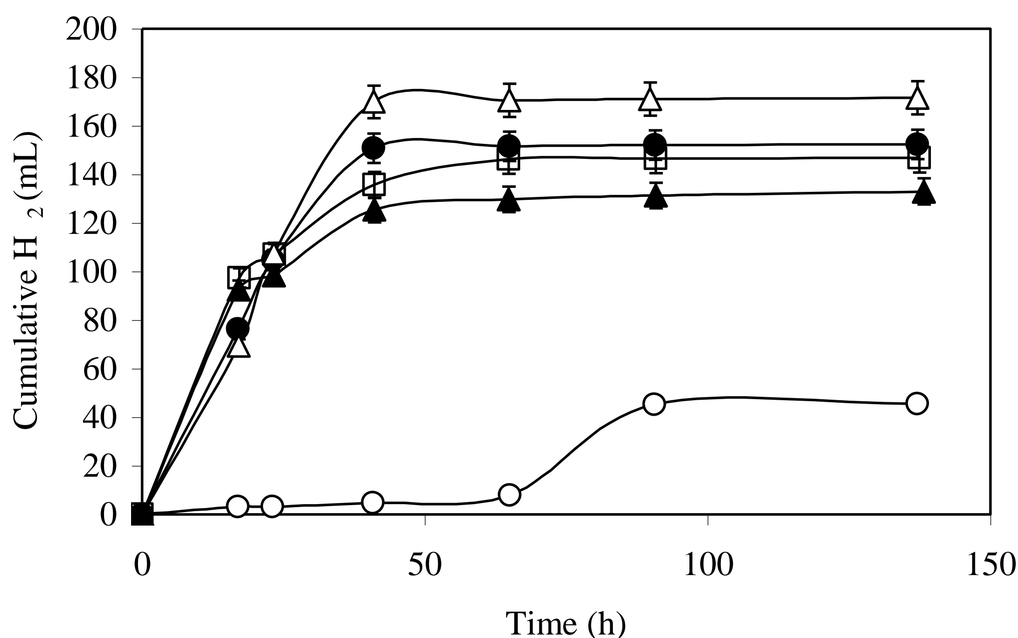


Figure 5.16 Variation of cumulative hydrogen gas with time for different initial cell concentrations ○ 0.5 g L^{-1}, ● 1.5 g L^{-1}, Δ 2.5 g L^{-1}, ▲ 3.5 g L^{-1}, □ 5 g L^{-1}

Cumulative hydrogen data were correlated with the Gompertz equation to determine the Gompertz coefficients by regression analysis as explained in section 0. Table 5.14 summarizes the Gompertz equation coefficients for different initial biomass concentrations. The highest cumulative hydrogen (172.5 mL) and the formation rates (7.76 mL h^{-1}) were obtained with an initial biomass concentration of 2.5 gL^{-1}. The lag phase decreased steadily with increasing the initial biomass concentration with a minimum lag phase of 1.68 h at X_o = 3.5 gL^{-1}. High initial biomass concentrations decreased the lag phase due to high biomass/substrate ratio. However, the rate and the extent of of hydrogen formation was maximum at X_o = 2.5 gL^{-1} corresponding an optimum Xo/So ratio of 0.125 g biomass g^{-1} WP. Probably, high biomass concentrations resulted in floc formation which reduced substrate-biocatalyst contact surface area resulting in low hydrogen production rates. Hydrogen may be consumed for acetic acid formation by the homo-acetogenic bacteria or high VFA concentrations obtained at high biomass concentrations may have caused product inhibition on hydrogen formation.

Table 5.14 Gompertz eqn. coefficients for different initial biomass concentrations (WP_o=20 gL^{-1})

Initial biomass concentration (gL^{-1})	P (mL)	R_m (mL h^{-1})	λ (h)	R^2
0.5	45.54	3.71	63.03	0.98
1.5	153.15	6.53	5.55	0.99
2.5	172.57	7.76	8.38	0.99
3.5	130.06	5.80	1.68	0.98
5.0	144.68	5.99	1.66	0.99

The initial and the final concentrations of WP-starch and TVFA are summarized in Table 5.15 for different initial cell concentrations. More than 85% of the starch content of wheat powder was hydrolyzed. Final VFA concentration was the lowest with the cell concentration of 2.5 gL^{-1} yielding the highest hydrogen formation. High TVFA concentrations produced at cell concentrations above 3 gL^{-1} resulted in low hydrogen formations due to product (VFA) inhibition. The rate and the extent of hydrogen production was inversely related with the final VFA concentration. High VFA concentrations were obtained due to a shift in metabolic pathway towards VFA formation which caused product inhibition for hydrogen formation.

Table 5.15 Initial and final starch and TVFA concentrations for the experiment with variable cell concentration

Initial cell concentration (gL^{-1})	$Starch_o$ (gL^{-1})	$Starch_F$ (gL^{-1})	$TVFA_o$ (gL^{-1})	$TVFA_F$ (gL^{-1})
0.5	17.97	2.28	0.53	6.6
1.5	17.43	1.9	0.53	3.76
2.5	17.03	1.94	0.53	3.43
3.5	17.98	2.27	0.53	6.28
5	17.28	2.40	0.53	6.53

The yield coefficients of hydrogen (mL H_2 g^{-1} starch) and VFA (mg VFA g^{-1}starch) varied with the initial biomass concentration as shown inFigure 5.17. Hydrogen yield coefficient (mL H_2 g^{-1} starch) increased with the initial biomass concentration up to 2.5 g L^{-1} and reached the highest level (57.1 mL H_2 g^{-1}starch, at 30 ^oC, 1 atm). Further increases in initial biomas to 5 gL^{-1} resulted in a lower hydrogen yield of 49 mL H_2 g^{-1} starch (30 ^oC, 1 atm) for the initial WP of 20 g L^{-1}. Unlike hydrogen yields, TVFA yields decreased with the initial biomass up to 2.5 gL^{-1} (192 mg VFA g^{-1} starch) and then increased with further increases in biomass

concentration up to 5 gL^{-1}. The highest TVFA yield (403 mg VFA g^{-1} starch) was obtained at the highest cell concentration of 5 gL^{-1} probably due to acetic acid formation by the homo-acetogenic bacteria. TVFA yield was minimum when the hydrogen yield was maximum. This behavior indicates changes in the metabolic pathways depending on the initial cell concentration and the composition of the anaerobic bacteria present in the microbial flora. The optimum biomass concentration resulting in the highest hydrogen and the lowest VFA yields was 2.5 g L^{-1} corresponding an optimal initial biomass/substrate ratio of Xo/So = 0.125 g biomass g^{-1} starch.

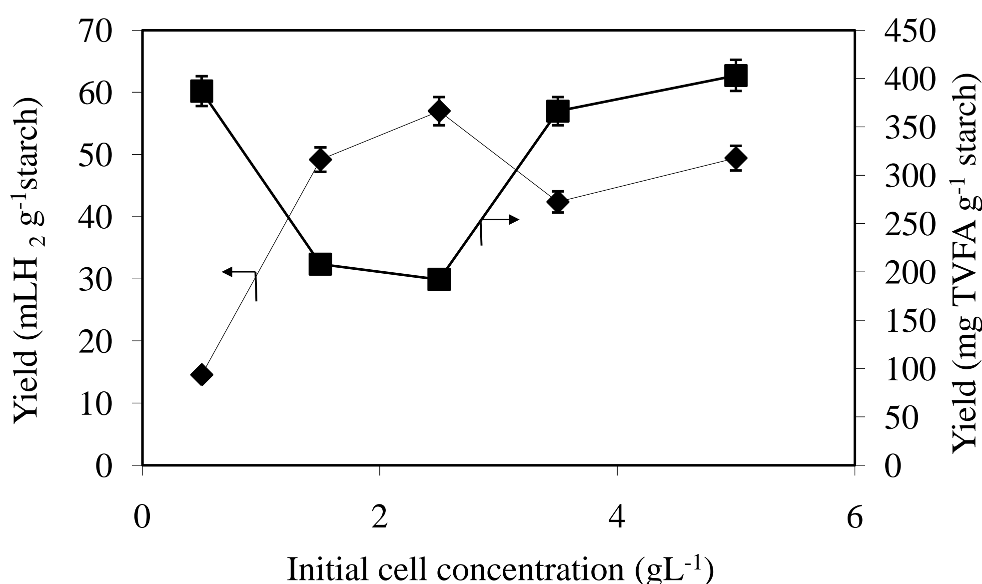


Figure 5.17 Variation of hydrogen (♦) and TVFA (■) yields with the initial cell concentration.

Specific hydrogen production rates (SHPR, mL H_2 g^{-1}biomass h^{-1}, at 30 °C , 1atm) were calculated from the Rm (mL h^{-1}) values of the Gompertz equation as explained in section 0Variation of SHPR's with the initial biomass concentration is depicted in Figure 5.18. SHPR decreased steadily with the initial cell concentration since SHPR was calculated by dividing the volumetric rate to the amount of biomass as shown in section 0. The highest SHPR (37 mL H_2 g^{-1}biomass h^{-1}) was obtained with the initial cell concentration of 0.5 gL^{-1}.

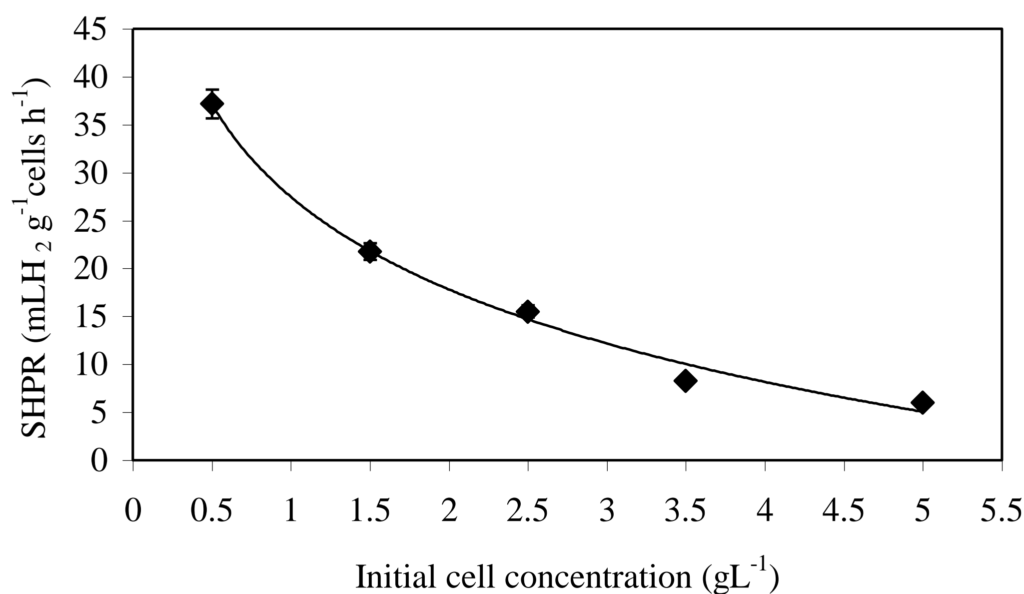


Figure 5.18 Variation of specific hydrogen production rate with the initial cell concentration.

Although the hydrogen yields obtained in this study are lower than the theoretical yields due to mixed VFA formation and consumption of hydrogen by homo-acetogens, our results are comparable with the literature reports in terms of the optimal starch concentration and the hydrogen yield (Lee et al., 2008). The experimental data obtained at different WP concentrations (Figure 5.15) were used to determine the kinetic constants by regression analysis according to section 4.1.3.1. The Quasi-Newton approximation yielded the values for the constants as k = 49.3 mL H_2 g^{-1}biomass h^{-1}, Ks = 39.66 gL^{-1}, K_I = 39.7 gL^{-1}. The inhibition constant (K_I) is the substrate concentration at which the reaction rate is reduced to one-half of its maximum value. Therefore, the initial WP-starch concentration must be below 40 gL^{-1} in order to obtain high hydrogen formation rates.

5.1.2 Batch Experiments of Light Fermentation

5.1.2.1 Microbial Culture Selection

In order to select the most appropriate bacterial culture for light fermentative hydrogen production from dark fermentation effluent (DFE), two sets of experiments

were carried out. The first set of experiment was carried out with different pure cultures of *Rhodobacter sphaeroides* (RS) namely the RS-RV, RS-NRRL and RS-DSMZ. In the second set of experiment, combinations of *Rhodobacter* species were used in order to select the best combination yielding the highest hydrogen formation rate and the yield. The same dark fermentation effluent containing 2400 ± 200 mgL^{-1} TVFA and 3.6 mgL^{-1} NH_4-N was used as substrate.

5.1.2.1.1 Comparison of Performances of Pure Cultures. Figure 5.19 shows cumulative hydrogen formation for three different *Rhodobacter sphaeroides* (RS) species. The RS-DSMZ culture produced less than 5 mL hydrogen while the volumes of cumulative hydrogen produced by the RS- NRRL and RS-RV were 48 mL and 32 mL, respectively at the end of 180 h incubation period. Hydrogen production by RS-RV culture was much faster than the others reaching the final level of 32 mL within 130 hours.

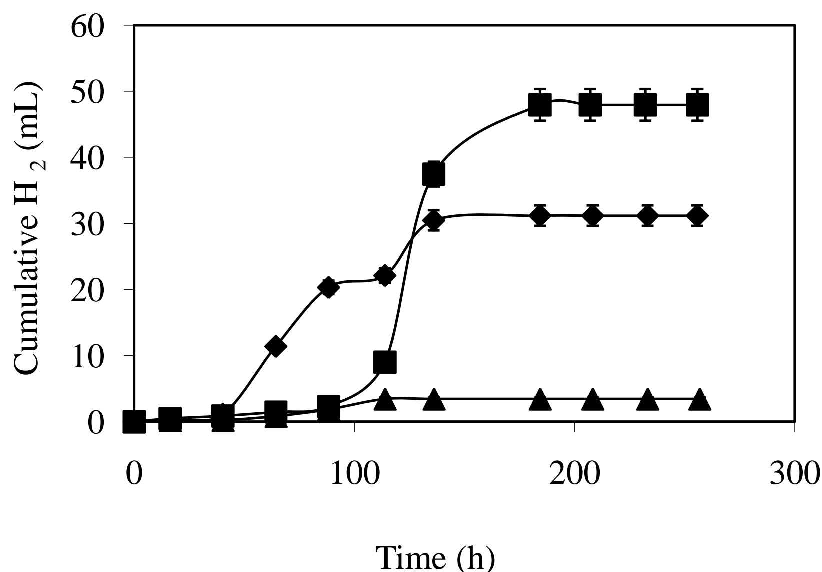


Figure 5.19 Variation of cumulative hydrogen formation with time for different pure *Rhodobacter sphaeroides* cultures (▲) DSMZ, (♦) RV, (■) NRLL. ($TVFA_o$ = 2400 mgL^{-1}, NH_4-N_o = 3.6 mgL^{-1})

Cumulative hydrogen data depicted in Figure 5.19 were correlated with the Gompertz equation and the constants were determined by regression analysis as

explained in section 4.1.2. Table 5.16 summarizes the Gompertz equation coefficients for different *Rhodobacter* species.

Table 5.16 Gompertz equation constants for different pure *Rhodobacter sphaeroides* cultures ($TVFA_o$ = 2400 mgL^{-1}, NH_4-N_o = 3.6 mgL^{-1})

Type of culture	P (mL)	R_m (mL h^{-1})	λ (h)	R^2
RV	31.45	0.39	37.73	0.98
NRLL	47.80	1.52	108.26	0.99
DSMZ	3.52	0.065	53.95	0.98

As presented in Table 5.16, the highest cumulative hydrogen (47.8 mL) and formation rate (1.52 mL h^{-1}) were obtained with the RS-NRRL culture. However, the lag phase was the lowest (37.7 h) with the RS-RV culture. Table 5.17 summarizes hydrogen yields and SHPR's obtained with different pure *Rhodobacter* cultures. The highest hydrogen yield (249 mL H_2 g^{-1} TVFA at 30 °C and 1 atm) was obtained with the RS-RV strain since TVFA consumption was low (0.125 g TVFA) for this strain as compared to the others. Theoretical maximum yield is 1630 mL H_2 g^{-1} acetic acid at STP (O °C, 1 atm) when acetic acid is the only VFA. The reason for low hydrogen yields is mainly the presence of a mixture of VFAs and also utilization of part of VFAs for growth and maintenance. The highest SHPR (13.8 mL H_2 g^{-1} biomass h^{-1}) was obtained with the RS-NRRL strain since the cumulative hydrogen formation was the highest with this strain. The hydrogen yield and SHPR obtained with the RS-DSMZ culture were not satisfactory. On the basis of the pure culture experiments, it can be said that either RS-NRRL or RS-RV cultures or a combination of both should be used for effective hydrogen production by the light fermentation of the dark fermentation effluent of the WP solution.

Table 5.17 The hydrogen yields and SHPR for different pure *Rhodobacter sphaeroides* cultures
The hydrogen yield for the dark fermentation of 1.53 molH_2 mol^{-1}glucose was added to the light fermentation yield to obtain the total yield.

Type of culture	$TVFA_o$ (mgL^{-1})	$TVFA_F$ (mgL^{-1})	Yield (mL H_2 g $TVFA^{-1}$)	Total Yield (mol H_2 mol^{-1} glucose)	SHPR (mLH_2 g^{-1} biomass h^{-1})
RV	2340	1830	249.50	2.35	3.55
NRLL	2680	1380	150.70	2.02	13.80
DSMZ	2280	820	9.70	1.85	0.59

5.1.2.1.2 Comparison of Performances of Mixed Cultures. In order to determine the performance of mixed *Rhodobacter* cultures, different combinations of the *Rhodobacter* species were used for inoculation of the fermentation bottles. Figure 5.20 depicts variation of cumulative hydrogen formation for different combinations of *Rhodobacter* cultures. The mixed cultures performed much better than the pure cultures producing higher cumulative hydrogen volumes (90 mL) as compared to the pure cultures (48 mL). The dark fermentation effluent was a mixture of different VFAs mainly acetic, butyric and propionic acids. Different *Rhodobacter* species preferably used different VFAs yielding higher extent of hydrogen production from the mixture of VFAs when mixtures of *Rhodobacter* species were used.

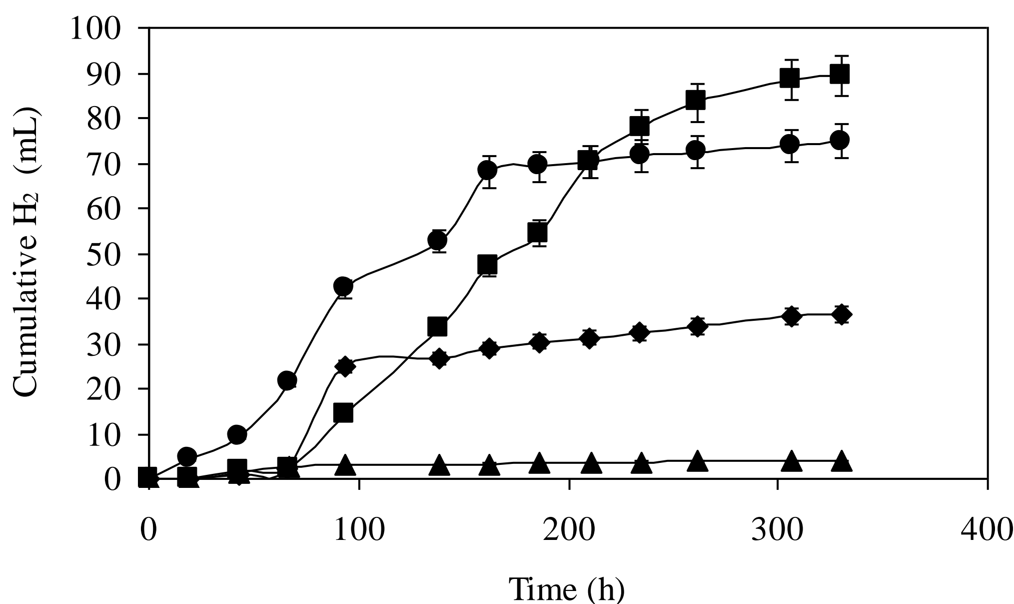


Figure 5.20 Variation of cumulative hydrogen formation with time for different combinations of *Rhodobacter sphaeroides* cultures (♦) RV + NRRL, (▲) NRRL + DSMZ, (■) RV + DSMZ, (●) RV + NRRL + DSMZ.

The highest cumulative hydrogen formation (90 mL in 330 h) was obtained with the combination of RV + DSMZ strains. However, hydrogen formation with this combination was slower as compared to the combination of the three strains. Hydrogen formation with the combination of the three *Rhodobacter* species was much faster than the others yielding 70 mL hydrogen within 150 h. Hydrogen

formation with the mixture of RV and DSMZ cultures was only 45 mL within the first 150 h, which increased further later on. The mixture of RV and NRRL cultures produced 32 mL H_2 within 150 h. The highest hydrogen formation rate (0.976 mL h^{-1}) was obtained with the combination of RV and NRRL cultures.

Table 5.18 summarizes the Gompertz equation constants for cumulative hydrogen production by different combinations of *Rhodobacter* cultures. The highest cumulative hydrogen formation (96 mL) was obtained with the mixed culture of RV and DSMZ while the highest hydrogen formation rate (0.976 mL h^{-1}) was obtained with a mixture of RV and NRRL cultures. Hydrogen formation rates for the other combinations were much lower than this (0.55 mL h^{-1} for RV +DSMZ and 0.596 mL h^{-1} for the combination of the three cultures). Probably, the RV and NRRL species preferably used the same VFAs yielding high rates, but low extent of hydrogen formation. A mixture of the NRRL and DSMZ cultures yielded the lowest cumulative hydrogen of less than 5 mL indicating the unfavorable interaction among the two species. In order to obtain high cumulative hydrogen and formation rate either RV+ DSMZ or RV+NRRL mixtures should be used. Apparently, inclusion of RV culture has improved the performance of the mixtures considerably. The lag phase was low (28 h) with the combination of the three cultures.

Table 5.18 Gompertz equation coefficients for different combinations of *Rhodobacter* cultures

Type of culture	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
RV+ NRRL	32.03	0.98	65.14	0.97
RV+DSMZ	95.94	0.55	76.96	0.99
NRRL+DSMZ	3.59	0.056	20.14	0.96
RV+NRRL+DSMZ	74.71	0.60	28.27	0.99

The initial and final TVFA concentrations and hydrogen yields are summarized in Table 5.19 for different mixtures of the *Rhodobacter* cultures. A mixture of the three cultures resulted in the highest yield of 693 mL H_2 g^{-1}TVFA which is lower than the theoretical maximum yield of 1630 mL H_2 g^{-1} TVFA at STP when acetic acid was used as the sole VFA. Part of the reason for this difference is the presence of a mixture of organic acids in the fermentation media and also utilization of VFAs for growth and/or PHB formation. The hydrogen yield obtained with the mixture of the RV and DSMZ cultures (663 mL H_2 g^{-1}TVFA) was comparable with the highest

yield. However, this yield is higher than that obtained with the pure *Rhodobacter* species. Therefore, organic acids were used more efficiently for hydrogen production when a mixed culture was used. The dark fermentation yield of 1.53 mol H_2 mol^{-1}glucose was added to the light fermentation yield to obtain the total hydrogen yield. The highest total hydrogen yield was obtained with the combination of the three cultures (3.81 mol H_2 mol^{-1}glucose). A mixture of RV and DSMZ cultures resulted in a yield of 3.72 mol H_2 mol^{-1} glucose. Unlike the other mixtures, the highest SHPR (19.85 mL H_2 g^{-1} biomass h^{-1}) was obtained with the combination of RV and NRRL cultures. The SHPR obtained with the mixture of the three cultures was 12.1 mL H_2 g^{-1} biomass h^{-1}. Considering the aforementioned results, a mixture of the three *Rhodobacter* species was used for future light fermentation experiments.

Table 5.19 The hydrogen yields and SHPR for different combinations of the *Rhodobacter sphaeroides* cultures. The hydrogen yield for the dark fermentation was 1.53 mol H_2 mol^{-1} glucose which was added to the light fermentation yield to obtain the total yield.

Combination of cell cultures	$TVFA_o$ (mgL^{-1})	$TVFA_F$ (mgL^{-1})	Yield (mL H_2 g^{-1} TVFA)	Total Yield (mol H_2 mol $^{-1}$ glucose)	SHPR (mL H_2 g^{-1}biomass h^{-1})
RV+ NRRL	2250	810	103.20	1.87	19.85
RV+DSMZ	1960	1410	662.90	3.72	11.16
NRRL+ DSMZ	1970	890	14.90	1.58	1.14
RV+NRRL+D SMZ	1700	1260	692.80	3.81	12.11

5.1.2.2 Effects of Initial TVFA and NH_4-N Concentrations

Effects of initial TVFA and NH_4-N concentrations on hydrogen formation rate and yield were investigated by using a mixture of the three *Rhodobacter* cultures. The dark fermentation effluent was diluted with pure water to obtain initial TVFA concentrations between 1200 and 5800 mgL^{-1} and NH_4-N concentrations between 18 and 185 mg L^{-1} . Figure 5.21 depicts variation of cumulative hydrogen formation with time for different initial TVFA concentrations. Cumulative hydrogen formation increased with increasing TVFA concentration up to 2350 mgL^{-1} and reached the highest level of 63.5 mL indicating substrate limitations at low TVFA

concentrations. High initial TVFA concentrations above 2350 mgL^{-1} resulted in low cumulative hydrogen formation due to substrate inhibition caused by high concentrations of TVFA. The most suitable initial TVFA concentration was 2350 ± 50 mgL^{-1} maximizing the cumulative hydrogen formation. This finding is in agreement with the literature results (Chen et al., 2007; Lee et al., 2007; Yokoi et al., 1998).

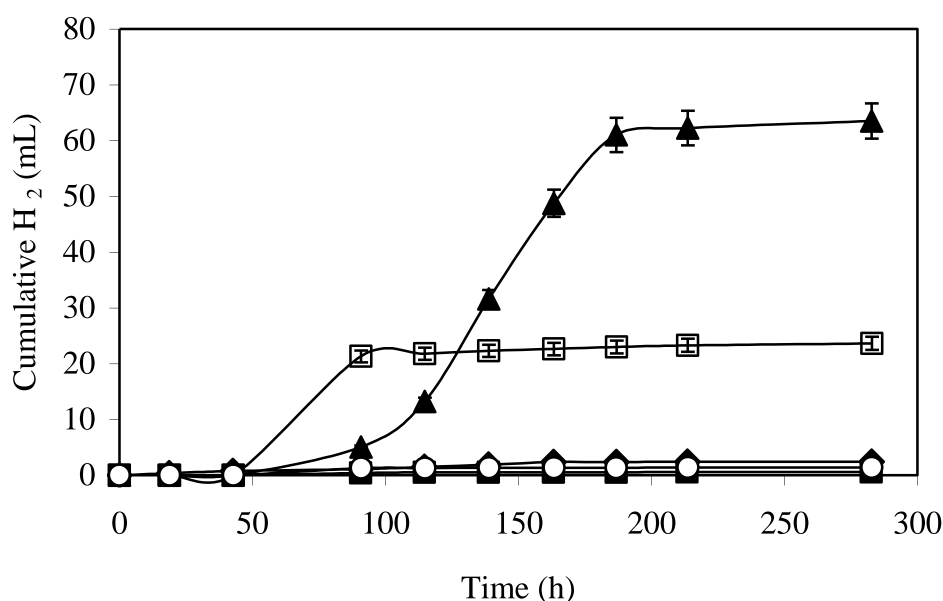


Figure 5.21 Cumulative hydrogen formation by the light fermentation of the dark fermentation effluent with different initial TVFA concentrations. $TVFA_o$ (mgL^{-1}): (♦) 5795, (■) 2930, (▲) 2350, (□) 1490, (○) 1200. A mixture of the three *Rhodobacter* cultures was used.

Table 5.20 summarizes the data on TVFA utilization, and also on hydrogen formation rates and yields for different initial TVFA concentrations. Since a different effluent was used for this set of experiments, the hydrogen yield of the dark fermentation in this case was 0.65 mol H_2 mol $glucose^{-1}$, which was added to the light fermentation yield to obtain the total yield. Hydrogen yield of the light fermentation was the highest (308 mL H_2 g^{-1}TVFA) with an initial TVFA concentration of 2350 mgL^{-1}. TVFA concentrations below and above 2350 mgL^{-1} resulted in lower hydrogen yields due to substrate limitation and inhibition, respectively. Total hydrogen yield of dark and light fermentations was also

maximum (1.41 mol H_2 mol^{-1} glucose) with the initial TVFA of 2350 mgL^{-1}. SHPR also increased with increasing TVFA concentration (substrate limitation) and reached the highest level (8.2 mL H_2 g^{-1}biomass h^{-1}) at $TVFA_o$ = 2350 mgL^{-1} which decreased considerably with further increases in TVFA due to substrate inhibition. On the basis of hydrogen formation rates and the yields, the initial TVFA of 2350 mgL^{-1} was also the optimum.

Table 5.20 The hydrogen yields and formation rates for different initial TVFA concentrations. The hydrogen yield of the dark fermentation was 0.65 mol H_2 mol^{-1} glucose which was added to the light fementation yield to obtain the total yield. A mixture of *Rhodobacter sphaeroides* NRRL + RV + DSMZ was used for all experiments.

$TVFA_o$ (mgL^{-1})	**$TVFA_F$ (mgL^{-1})**	**Yield (mL H_2 g TVFA^{-1})**	**Total Yield (mol H_2 mol^{-1} glucose)**	**SHPR (mL H_2 g^{-1} biomass h^{-1})**
5795	4480	9.70	0.67	0.15
2930	2450	6.20	0.66	0.09
2350	1250	308.30	1.41	8.19
1490	450	121.20	0.94	7.04
1200	123	6.80	0.66	0.41

Table 5.21 summarizes Gompertz equation coefficients for different initial TVFA concentrations in the light fermentation. Consistent with the experimental findings, the maximum hydrogen formation rate (0.85 mL H_2 h^{-1}) and the volume (65.5 mL) were obtained with the initial TVFA of 2350 mgL^{-1}. However, the lag phase was somewhat high (99 h) for this TVFA concentration.

Table 5.21 Gompertz equation coefficients for different initial TVFA concentrations. A mixture of *Rhodobacter sphaeroides* RV, NRLL and DSMZ was used for all experiments.

$TVFA_o$ (mgL^{-1})	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
5795	2.63	0.01	7.14	0.97
2930	0.54	0.01	53.73	0.99
2350	65.55	0.85	99.44	0.99
1490	22.84	0.72	50.02	0.99
1200	1.33	0.04	50.27	0.99

Variations of the rate and cumulative hydrogen formation with the initial TVFA concentration are depicted in Figure 5.22. The highest cumulative hydrogen and formation rate was obtained with the initial TVFA of 2350 mgL^{-1}. Substrate limitations and inhibitions were observed for TVFA concentrations below and above

2350 mgL^{-1}, respectively. Negligible amount (0.45 mL) of cumulative hydrogen gas was produced in the control flask.

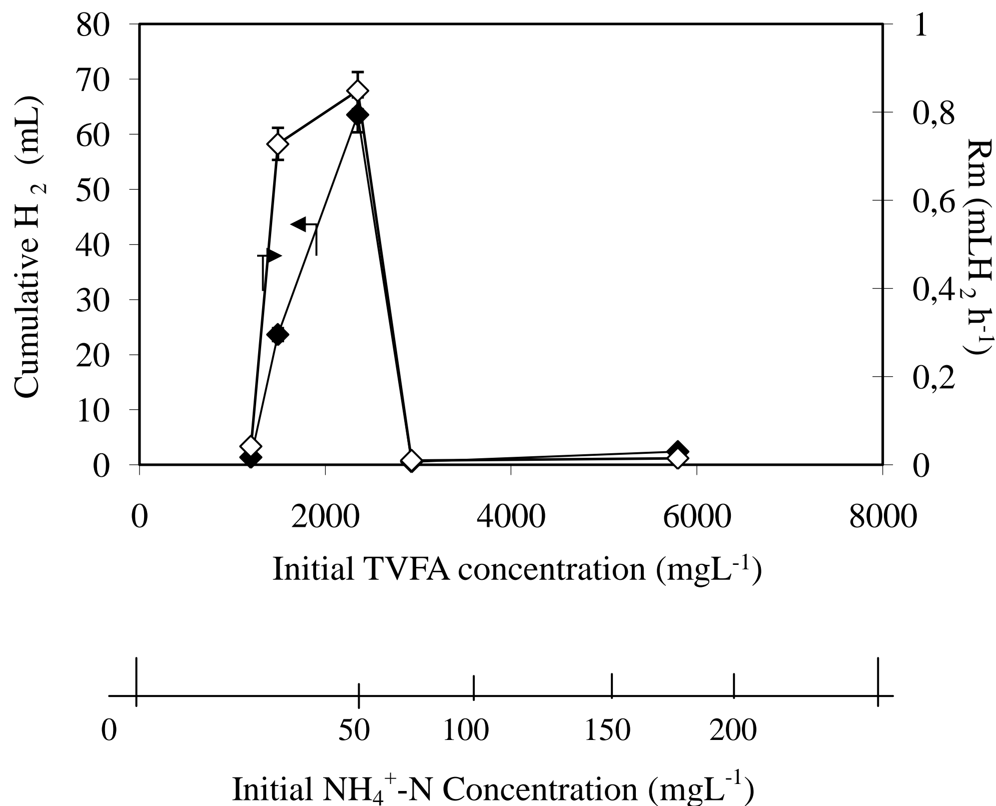


Figure 5.22 Variation of cumulative hydrogen and formation rate with the initial TVFA and NH_4-N concentrations. (♦) Cumulative hydrogen (mL), (◊) Maximum hydrogen production rate (mL H_2 h^{-1}).

Since the light fermentation media with different TVFA concentrations were obtained by dilution of the dark fermentation effluent, the initial NH_4-N concentrations also varied for different fermentation media. High NH_4-N concentrations above 40 mgL^{-1} was reported to be inhibitory for the nitrogenase enzyme used for hydrogen production during Light fermentation (Yokoi H., *et al.*, 1998). In order to determine the ammonium ion inhibition on hydrogen formation, the rate and cumulative hydrogen formation (CHF) were plotted against initial NH_4-N concentration in Figure 5.22. Just like variation of CHF with TVFA concentrations, CHF increased with initial NH_4-N concentration up to 47 mgL^{-1} due to limitation by the ammonium ions and became maximum (63.5 mL H_2) at an initial

NH_4-N of 47 mgL^{-1}. Further increases in the initial NH_4-N decreased the rate and the extent of hydrogen formation due to inhibition of the nitrogenase enzyme by high NH_4-N concentrations.

Our results are in agreement with the studies reporting inhibitions caused by high TVFA and NH_4-N concentrations (Chen et al., 2007; Lee et al., 2007; Yokoi et al., 1998). The TVFA and NH_4-N concentrations maximizing the hydrogen formation were reported to be approximately 2000 mgL^{-1} and 30 mgL^{-1}, respectively (Barbosa et al., 2001; Chen et al., 2007; Lee et al., 2007; Yokoi et al., 1998). The optimum TVFA and NH_4-N concentrations determined in this study (2350 mgL^{-1} and 50 mgL^{-1}, respectively) are somewhat above the literature reports. This is probably due to utilization of a mixed *Rhodobacter* cultures with different TVFA toleration limits in the light fermentation.

5.1.2.3 Effects of Initial Biomass Concentration

Effects of initial biomass concentration on light fermentative hydrogen gas production was investigated by varying the initial cell concentration between 0.5-5 gL^{-1}. Figure 5.23 presents the variation of cumulative hydrogen gas with time for different initial cell concentrations. As shown in Figure 5.23 for almost all conditions a long Lag time occurred. Hydrogen production was observed with 0.5 gL^{-1} biomass concentration after 90h fermentation. Other flasks began to produce hydrogen after about 115 h. The highest cumulative hydrogen (71 mL) was obtained with 0.5 gL^{-1} biomass concentration which decreased with increasing initial cell concentration. Cumulative hydrogen production was apparently low for initial cell concentrations higher than 1 gL^{-1}. Probably this was a result of bacterial floc formation causing substrate diffusion limitations inside flocs and also light penetration problems through the fermentation media. Although the volume of cumulative hydrogen leveled off after about 180h, the flask with 0.5 gL^{-1} began to produce additional hydrogen at 220 h which leveled off at 280 h of fermentation. Negligible amount of hydrogen was produced in the control flask where no bacterial inoculation was done.

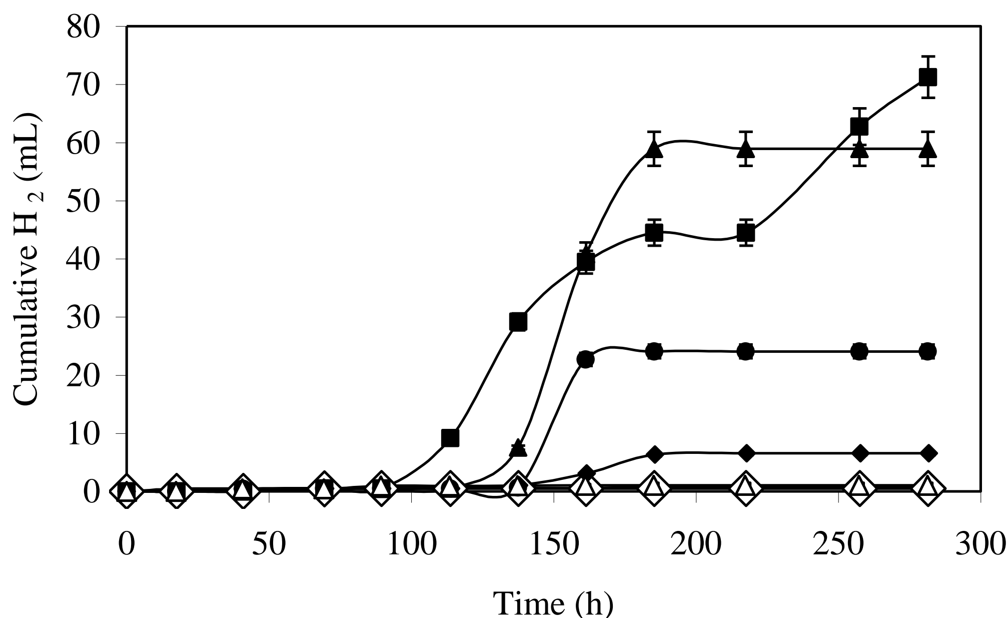


Figure 5.23 Variation of cumulative hydrogen gas with time for different initial biomass concentrations (◊) Control, (■) 0.5 gL^{-1}, (▲) 1gL^{-1}, (●)1.5 gL^{-1}, (♦) 2.5 gL^{-1}, (□) 3.5 gL^{-1}, (Δ) 5gL^{-1}

The data obtained in Figure 5.23 were correlated with the Gompertz equation as explained in section 0and the constants were determined by regression analysis. Table 5.22 presents the Gompertz equation constants. According to Table 5.22 maximum hydrogen production potentials was obtained for low initial biomass concentrations. The highest and lowest hydrogen production potentials were 71.42 mL, 0.63 mL for 0.5 gL^{-1} and 3.5 gL^{-1}, respectively. Biomass concentration of 5 gL^{-1} had almost the same hydrogen production potential with 3.5 gL^{-1}. The same trend was valid for the maximum hydrogen production rates (R_m). High rates were observed at low initial biomass concentrations. The R_m value was maximum for 1gL^{-1} and minimum for 5gL^{-1}. Although the highest hydrogen potential was observed with 0.5 gL^{-1}, higher rates were obtained with 1 and 1.5gL^{-1} compared to 0.5 gL^{-1}. Even very low lag durations were observed for 3.5 gL^{-1} (0.06h) and 5 gL^{-1} (14.98h) initial cell concentrations, negLigible amount of hydrogen was produced with respect to low rates for those bottles compared to the other initial cell concentrations. Lag time increased with increasing the cell concentration simultaneously from 88.38h to 139.1 h then remained almost constant.

Table 5.22 Gompertz equation constants for different initial cell concentrations in light fermentation.

Biomass Concentration (gL^{-1})	**P (mL)**	**R_m ($mL\ h^{-1}$)**	**λ (h)**	**R^2**
0 (Control)	0.51	0.02	40.98	0.99
0.5	71.42	0.88	88.38	0.97
1	59.61	1.66	113.64	0.99
1.5	24.08	1.52	139.10	0.99
2.5	6.70	0.14	134.88	0.98
3.5	0,63	0.02	0.06	0.78
5	1.06	0.01	14.98	0.95

The initial and final TVFA concentrations, hydrogen yields and SHPR values are summarized in Table 5.23 for different initial biomass concentrations. TVFA was consumed in all bottles including the control, which probably was subjected to contamination. Nearly 50% TVFA consumption was observed for 0.5 gL^{-1} which resulted in the highest yield and SHPR. TVFA consumptions of about 58% to 73% were obtained for 1.5 and 2.5 gL^{-1} initial cell concentrations where hydrogen formation yields were lower compared to 0.5 gL^{-1} and 1 gL^{-1}. This might be a result of the utilization of the TVFA for other cellular and deposition materials at high cell concentrations instead of producing hydrogen gas. The highest yield was obtained with 0.5 gL^{-1} initial biomass concentration as 387 mL H_2 g^{-1}TVFA which is lower than the theoretical maximum yield of 1630 mL H_2 g^{-1} TVFA at STP when acetic acid was used as the sole VFA. Part of the reason for this difference is the presence of a mixture of organic acids in the fermentation media and also utilization of VFAs for growth and/or PHB formation. The hydrogen yield obtained with 1 gL^{-1} initial cell concentration (340.61 mL H_2 g^{-1}TVFA) was comparable with the highest yield. The dark fermentation yield of 0.34 mol H_2 mol^{-1}glucose was added to the light fermentation yield to obtain the total hydrogen yield. The highest total hydrogen yield was obtained with 0.5 gL^{-1} initial cell concentration (1.80 mol H_2 mol^{-1}glucose) followed by the 1gL^{-1} cell concentration yield as 1.62 mol H_2 mol^{-1}glucose. The reason of such low yields is the low yield contribution from dark fermentation which actually is expected to be between 1.5-1.9 mol H_2 mol^{-1}glucose under normal conditions. A reason for that might be the use of natural microflora from the WPS instead using anaerobic sludge inoculum to obtain higher yields. SHPR values were in agreement with the hydrogen formation yields. The highest SHPR values were

10.66 mL H_2 g^{-1} biomass h^{-1} and 10.06 mL H_2 g^{-1} biomass h^{-1} for 0.5 and 1 gL^{-1} cell concentrations, respectively. SHPR values for initiail cell concentrations higher than 2.5 gL^{-1} resulted quite low SHPR when compared with lower initial cell concentrations. On the basis of the experimental results, initial biomass concentration between 0.5-1 gL^{-1} was selected as the most convenient cell concentrations for effective hydrogen production by light fermentation of dark fermentation effluent. The pH and ORP varied between 7.5-8.0 and -150mV and -200 mV, respectively through the fermentation. Since high biomass concentrations had adverse effects on light penetration through the reactor in light fermentation, initial biomass concentrations between 0.1-0.5 gL^{-1} were used in many studies in batch suspended experiments (Başar et al., 2007; Yokoi et al., 1998; Yokoi et al., 2000; Yokoi et al., 2002). Therefore, our results are in agreement with the literature. However high biomass concentrations in form of immobilized systems can be used in order to prevent any substrate inhibitions.

Table 5.23 Hydrogen yields, formation rates and TVFA consumptions for different initial biomass concentrations. The hydrogen yield of the dark fermentation was 0.34 mol H_2 mol^{-1} glucose which was added to the light fermentation yield to obtain the total yield. *Rhodobacter sphaeroides* -RV was used for all experiments.

Initial Biomass Concentration (gL^{-1})	**$TVFA_o$ (mgL^{-1})**	**$TVFA_F$ (mgL^{-1})**	**Yield (mL H_2 g^{-1} TVFA)**	**Total Yield (mol H_2.mol^{-1} glucose)**	**SHPR (mL H_2 g^{-1} biomass h^{-1})**
Control	2200	1312	3.49	0.35	-
0.5	2200	1084	387.22	1.80	10.66
1	2200	1151	340.61	1.62	10.06
1.5	2200	910	113.58	0.77	6.14
2.5	2200	589	24.67	0.43	0.34
3.5	2200	1870	13.70	0.39	0.03
5	2200	1877	20.13	0.41	0.01

5.1.2.4 Effects of Light source and Light Intensity

5.1.2.4.1 Effects of Light source. Six batch light fermentation experiments were performed with different light sources by using dark fermentation effluent of WPS as substrate. The light intensity was constant for all light sources at 270 W m^{-2}. Figure 5.24 depicts variation of cumulative hydrogen formation (CHF) with time for

different light sources. The highest CHF (252 mL) was obtained with halogen lamp and the lowest was with IR light (10.6 mL). Tungsten +IR light source yielded higher CHF (126 mL) as compared to the tungsten light alone (106 mL) indicating the contribution of IR light. Fluorescent and the sunlight yielded 103 mL and 74 mL CHF within 150 h. The uninoculated control experiment under the fluorescent light yielded negLigible CHF. Apparently, the halogen lamp was the most suitable light source among the others tested.

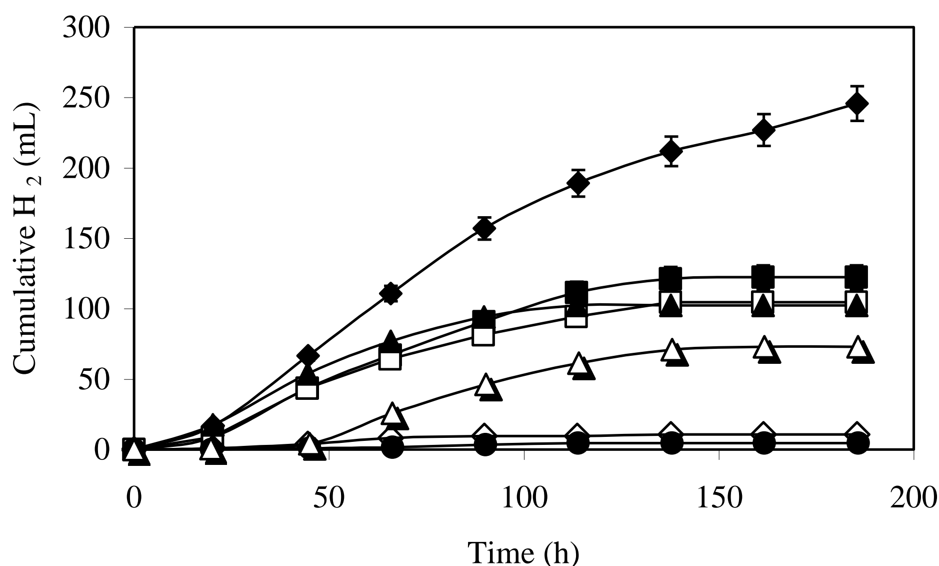


Figure 5.24 Variation of cumulative hydrogen formation with time for different light sources in light fermentation using pure *Rhodobacter sphaeroides*-RV culture (■) Tung + IR, (□) Tung, (◊) IR, (▲)Fluorescent, (♦) Halogen, (Δ) Sun, (●) Control.

Gompertz equation was used to correlate the cumulative hydrogen data depicted in Figure 5.24 and the constants were determined by regression analysis as explained in section 0.Table 5.24 presents the Gompertz equation coefficients for different light sources used. The maximum rate of hydrogen formation (R_m) varied between 0.19 and 2.18 mL h^{-1}. The highest rate was obtained with halogen lamp (2.18 mL h^{-1}) and the lowest with IR (0.19 mL h^{-1}). The rate and the extent of hydrogen formation showed the same trend for different light sources. The halogen lamp resulted in the highest rate and the extent of hydrogen gas formation. The lag phase varied between 10 and 20 h which is shorter than typical lag phases for the light fermentation.

Sunlight resulted in the longest lag period (43 h) probably due to varying Light intensities during the day.

Table 5.24 Gompertz equation constants for different light sources in light fermentation using pure *Rhodobacter sphaeroides*- RV culture ($TVFA_o$ = 2100 mg L^{-1}, NH_4-N_o = 4 mg L^{-1}).

Light Source	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
Tung	106.67	1.26	13.29	0.99
Tung & IR	126.57	1.41	16.61	0.99
IR	10.55	0.19	21.11	0.99
Fluorescent	103.83	1.57	10.42	0.99
Halogen	251.64	2.18	15.95	0.99
Sun	74.63	1.05	43.48	0.99

Table 5.25 summarizes hydrogen yields and specific hydrogen production rates (SHPR) for different light sources. Hydrogen yield of the light fermentation varied between 244 and 781 mL H_2 g^{-1} TVFA. The highest yield was obtained with halogen lamp (781 mL H_2 g^{-1} TVFA) and the lowest with IR (244 mL H_2 g^{-1} TVFA). Utilization of tungsten + IR light (681 mL H_2 g^{-1} TVFA) improved the hydrogen yield over the tungsten lamp alone (609 mL H_2 g^{-1} TVFA). Hydrogen yield for sun light (463 mL H_2 g^{-1} TVFA) was higher than that of the fluorescent light (377 mL H_2 g^{-1} TVFA) probably due to a wider range of wavelengths present in the sun light. Theoretical hydrogen yield in light fermentation of acetic acid is 4 moles H_2 mol^{-1} acetate which corresponds to 1650 mL H_2 g^{-1} acetate. The hydrogen yield obtained in this study (781 mL H_2 g^{-1} TVFA) is nearly 50% of the theoretical yield.The difference is due to the fact that the dark fermentation effluent contained a mixture of VFAs (acetic, butyric, propionic acids) and also part of the TVFA was used for growth, maintenance and other bacterial activities.

The hydrogen yield for the dark fermentation alone was 1.87 mol H_2 mol^{-1} glucose. Total hydrogen yield was calculated by addition of the dark and light fermentation yields after converting the light fermentation yield to mol H_2 mol^{-1} glucose. Similar to the CHF results the highest total hydrogen yield was obtained with halogen lamp (4.08 mol H_2 mol^{-1} glucose) which is nearly 1/3 of the theoretical total yield of 12 mol H_2 mol^{-1} glucose when acetic acid is the sole VFA produced by dark fermentation. As a result of formation of a mixture of VFAs and utilization of glucose and TVFA for growth and maintenance the yield was lower than the

theoretical. However, the highest total yield obtained in this study (4.08 mol H_2 mol^{-1} glucose) is comparable to those reported in literature (Table 5.28). The lowest total hydrogen yield was obtained with IR light (2.56 mol H_2 mol^{-1} glucose). Utilization of IR light in combination with the tungsten light improved the total hydrogen yield (3.79 mol H_2 mol^{-1} glucose).

Table 5.25 Hydrogen yields and SHPRs for different light sources in light fermentation using pure culture of *Rhodobacter sphaeroides*–RV. The hydrogen yield for the dark fermentation was 1.87 mol H_2 mol^{-1} glucose, which was added to the light fermentation yield to obtain the total yield.

Light Source	**$TVFA_o$ (mgL^{-1})**	**$TVFA_F$ (mgL^{-1})**	**Yield (mL H_2 g^{-1} TVFA)**	**Total Yield (molH$_2$mol^{-1} glucose)**	**SHPR (mLH$_2$ g^{-1} biomass h^{-1})**
Tung	2100	1413	609.88	3.59	10.12
Tung & IR	2100	1382	681.83	3.79	11.33
IR	2100	1926	244.13	2.56	1.50
Fluorescent	2100	1014	377.34	2.94	12.61
Halogen	2100	842	781.63	4.08	17.48
Sun	2100	1159	463.66	3.18	12.60

Specific hydrogen production rates (SHPR) based on the initial biomass concentration are also listed in Table 5.25. In agreement with the aforementioned results, the halogen lamp was found to be the most suitable light source yielding the highest SHPR (17.5 mL H_2 g^{-1} biomass h^{-1}). The IR light yielded the lowest SHPR (1.5 mLH$_2$ g^{-1} biomass h^{-1}). Maximum light absorption by the light fermentation bacteria *(Rhodobacter sp)* takes place at two distinct wave lengths of 522 nm and 860 nm (Akkerman I., *et al.*, 2002). Since the wavelength of IR light is above 750 nm, *Rhodobacter sp.* may not be able to absorb IR light efficiently. Halogen lamp is a light source with large range wavelengths including 522 and 860 nm yielding better light absorption by the *Rhodobacter* sp.

Figure 5.25 depicts variations of cumulative hydrogen (mL), TVFA (mg L^{-1}), glucose (gL^{-1}) and NH_4-N (mgL^{1}) concentrations with time for light fermentation using halogen lamp. TVFA concentration decreased from 2.1 gL^{-1} to 0.85 gL^{-1} within 180 h indicating effective breakdown of VFAs to CO_2 and H_2. The initial NH_4-N and glucose concentrations were 4 mgL^{-1} and 0.35 gL^{-1} which decreased to 0.4 mgL^{-1} and 0.1 gL^{-1}, respectively at the end of the light fermentation. Ammonium and glucose utilization indicated bacterial growth along with hydrogen gas formation. pH of the

medium increased from 7.0 to 7.4 due to removal of VFAs from the medium. ORP of the medium was -220 ± 40 mV.

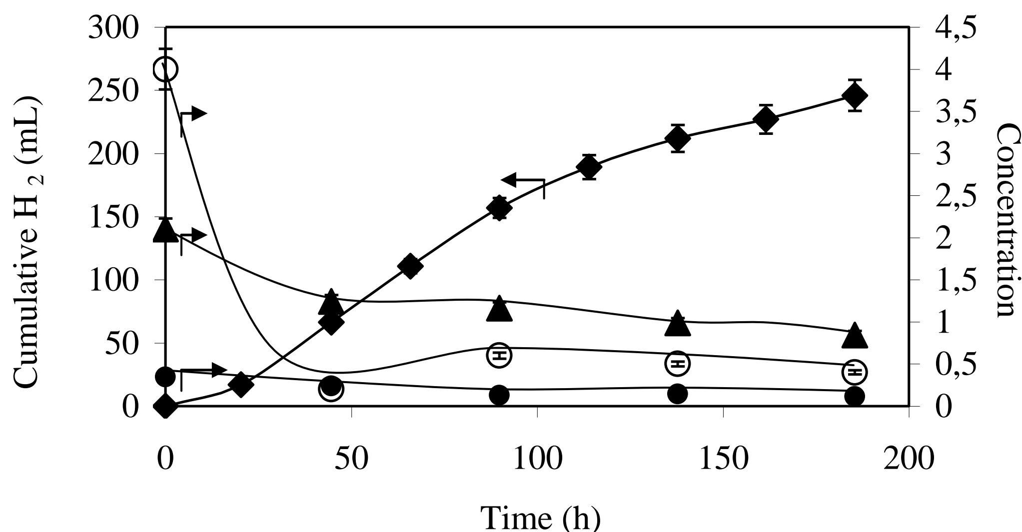


Figure 5.25 Variation of (♦) Cumulative hydrogen (mL), (▲) Total volatile fatty acid (gL^{-1}), (●)glucose (gL^{-1}) and (○) NH_4-N (mg L^{-1}) concentrations with time for the light fermentation using *Rhodobacter sphaeroides* –RV culture with halogen light source.

5.1.2.4.2 Effects of Light Intensity. In order to determine the most suitable light intensity with the highest yield and the extent of bio-hydrogen formation, halogen lamp was used at five different light intensities in light fermentation of the dark fermentation effluent using pure culture of *R. sphareoides*-RV. An experiment with the sun light and an uninoculated control experiment with halogen lamp were also used. Light intensities of the halogen lamp was varied between 1 and 10 klux by changing the distance between the light source and the serum bottles. Figure 5.26 depicts the variation of CHF with time for different light intensities and for the sun light.

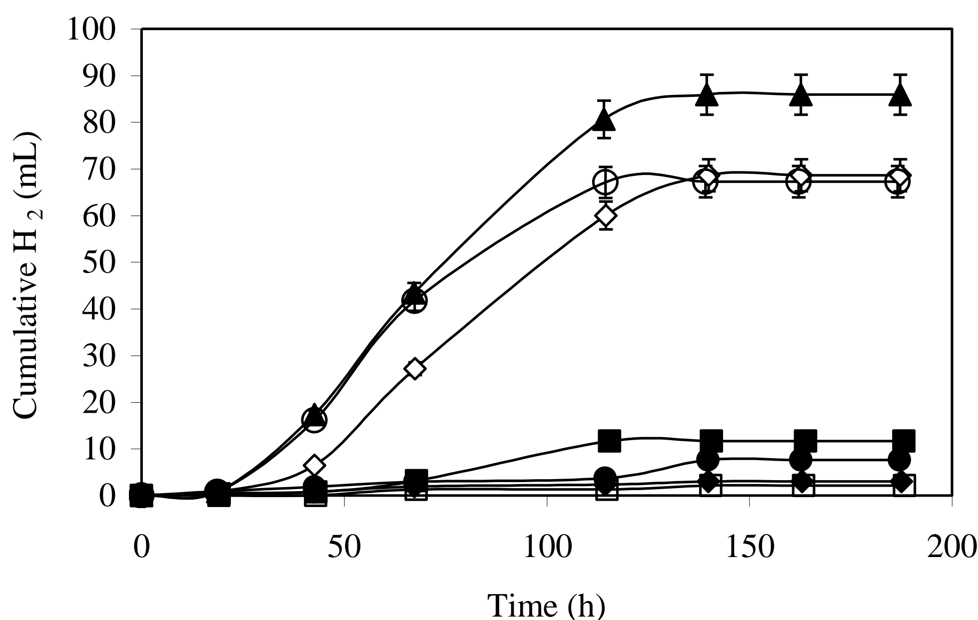


Figure 5.26 Variation of cumulative hydrogen formation with time for different light intensities in light fermentation using *Rhodobacter sphaeroides*-RV culture and halogen lamp. (♦) 1 klux, (◊) 3klux, (▲) 5klux, (o) 7klux, (●) 10klux, (■) Sun, (□) Control.

CHF increased with increasing Light intensity and reached the maximum level (88 mL) at 5 klux (approx. 176 Wm^{-2}) light intensity. Further increases in the light intensity above 5 klux resulted in decreases in CHF yielding 11 mL H_2 formation at 10 klux light intensity. Apparently availability of the light was the limiting factor in bio-hydrogen formation at low light intensities below 5 klux. Light inhibition of hydrogen formation was observed at light intensities above 5 klux. The optimum light intensity was 5 klux (176 Wm^{-2}). Light fermentation under the sunlight also yielded 10.5 mL H_2 within 140 h which was comparable with the 10 klux halogen lamp source.

The data presented in Figure 5.26 was correlated with the Gompertz equation and the constants were determined by regression analysis according to section 04.1.2. Table 5.26 summarizes the Gompertz equation constants for different light intensities. The highest rate (1.04 mL h^{-1}) and the extent (88 mL) of hydrogen formation took place with 5 klux light intensity. The lag phase varied between 16 and 40 h depending on the light intensity. In general, a decreasing tendency in the lag

phase was observed with the increasing Light intensity. High light intensities stimulated the initial hydrogen formation which became inhibitory for long term applications. The lag phases for 5 and 7 klux light intensities were 30 h. The sun light yielded longer (65 h) lag phase due to varying Light intensities during the day time.

Table 5.26 Gompertz equation constants for different light intensities in light fermentation using pure culture of *Rhodobacter sphaeroides*-RV ($TVFA_o$ = 1465 mg L^{-1}, NH_4-N_o= 2.16 mg L^{-1}).

Light Intensity (klux)	P (mL)	R_m (mL h^{-1})	λ (h)	R^2
1	2.90	0.05	33.92	0.95
3	70.44	0.69	40.12	0.99
5	88.07	1.04	30.62	0.99
7	68.35	0.70	29.62	0.99
10	10.63	0.15	16.20	0.93
Sun	11.66	0.18	65.27	0.99

The hydrogen yields and specific production rates (SHPR) are presented in Table 5.27 for different light intensities with halogen lamp. The amount of consumed TVFAs were not much different for different light intensities (400 ± 50 mgL^{-1}). However, effective utilization of TVFAs for hydrogen production varied with the intensity of light. The hydrogen yield of light fermentation increased from 43 mLH_2 g^{-1} TVFA at 1 klux to 1037 mLH_2 g^{-1} TVFA at 5 klux indicating Limitations by the availability of light at low light intensities. Further increases in the light intensity above 5 klux resulted in lower yields (103 mL H_2 g^{-1} TVFA at 10 klux) due to inhibition caused by high light intensities. Illumination with sunlight yielded 301 mL H_2 g^{-1} TVFA which is somewhat lower than the previously reported yield in Table 5.25 (463 mL H_2 g^{-1} TVFA) due to variations in the intensity of the sun light depending on the day. The hydrogen yield of 1037 mL H_2 g^{-1} TVFA obtained with halogen lamp at 5 klux intensity is about 63% of the theoretical yield (1650 mL H_2 g^{-1} TVFA) when acetic acid is the only substrate. This yield is also higher than the yield reported in Table 5.25 (781 mL H_2 g^{-1} TVFA). This is because the light intensity in the first set of experiments (270 W m^{-2}) was nearly 7.6 klux which was above the 5 klux optimum light intensity. In this set of expriments, the hydrogen yield for the dark fermentation alone was 1.52 mol H_2 mol^{-1} glucose. The light

fermentation yield was converted to mol H_2 mol^{-1} glucose and was added to the dark fermentation yield to obtain the total yield. The highest total hydrogen yield was obtained with 5 klux light intensity using halogen lamp (4.55 mol H_2 mol^{-1} glucose) which is nearly 40% of the theoretical total yield of 12 mol H_2 mol^{-1} glucose when acetic acid is the sole VFA produced by dark fermentation. The actual yield is always lower than the theoretical due to formation of a mixture of VFAs and utilization of glucose and TVFA for growth and maintenance. However, the highest total yield obtained in this study (4.55 mol H_2 mol^{-1} glucose) is comparable or higher than some of those reported in the literature as summarized in Table 5.28. The highest hydrogen yield obtained in the previous set of our experiments (4.08 mol H_2 mol^{-1} glucose at 270 Wm^{-2} or 7650 lux is almost the same as the hydrogen yield obtained in this set of experiments (4.04 mol H_2 mol^{-1} glucose at 7000 klux) indicating the reproducibility of the results.

Table 5.27 Hydrogen yields and SHPRs for different light intensities in the light fermentation using pure *Rhodobacter sphaeroides* –RV culture and halogen lamp. The hydrogen yield for the dark fermentation was 1.52 mol H_2 mol^{-1} glucose, which was added to the light fermentation yields to obtain the total yield.

Light Intensity (klux)	**$TVFA_o$ (mgL^{-1})**	**$TVFA_F$ (mgL^{-1})**	**Yield (mL H_2 g^{-1} TVFA)**	**Total Yield (molH_2mol^{-1} glucose)**	**SHPR (mLH_2 g^{-1} biomass h^{-1})**
1	1465	1114	43.22	1.65	0.37
3	1465	1007	749.23	3.71	5.46
5	1465	1051	1037.56	4.55	8.23
7	1465	1075	862.18	4.04	5.54
10	1465	1097	103.53	1.82	1.21
Sun	1465	1176	301.73	2.11	1.41

The SHPRs obtained with different light intensities are also presented in Table 5.27. Similar to CHF and yield, the SHPR also increased with increasing Light intensities up to 5 klux and decreased with further increases in the light intensity above 5 klux. The highest SHPR was obtained with 5 klux illuminaton (8.23 mL H_2 g^{-1} biomass h^{-1}) and the lowest was with 1 klux (0.37 mL H_2 g^{-1} biomass h^{-1}). The sunlight resulted in a SHPR of 1.41 mL H_2 g^{-1} biomass h^{-1} which is almost equal to the SHPR obtained with 9 klux light intensity. Apparently sunlight provided high light intensities causing Light inhibition on light fermentation

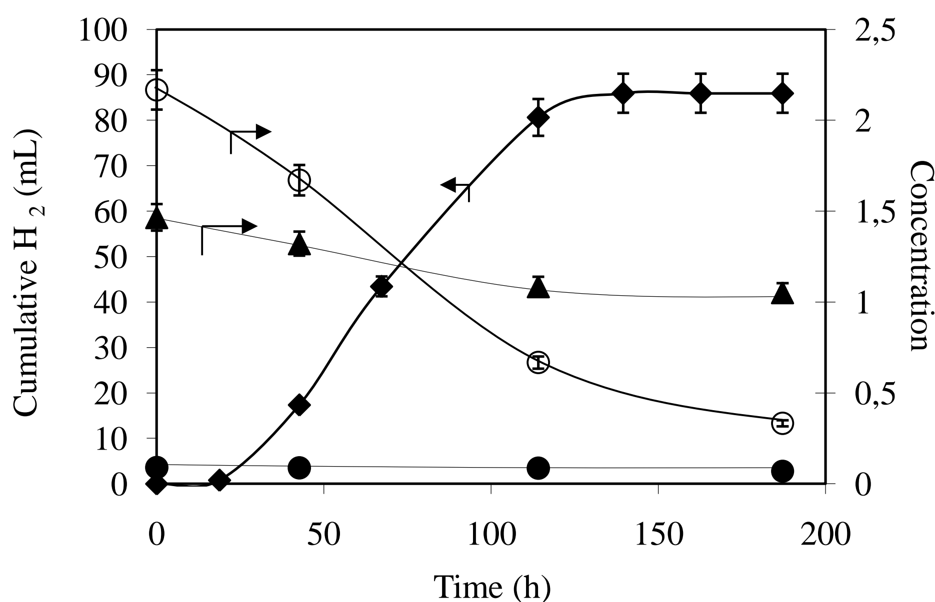


Figure 5.27 Variation of (♦) Cumulative hydrogen (mL), (▲) Total volatile fatty acid (gL^{-1}), (●)glucose (gL^{-1}) and (○) NH_4-N (mgL^{-1}) concentrations with time for the light fermentation using pure *Rhodobacter sphaeroides*–RV culture with halogen light source of 5 klux intensity.

Variations of cumulative hydrogen (mL), TVFA (g L^{-1}), glucose (gL^{-1}) and NH_4-N (mgL^{-1}) concentrations with time are depicted in Figure 5.27 for light fermentation with halogen lamp at 5 klux light intensity. TVFA concentration decreased from 1.465 gL^{-1} to 1.05 g L^{-1} within 185 h. The initial NH_4-N and glucose concentrations were 2.1 mgL^{-1} and 0.09 gL^{-1} which decreased to 0.33 mgL^{-1} and 0.07 gL^{-1}, respectively at the end of the fermentation period. Low initial ammonium and glucose concentrations facilitated light fermentation of VFAs producing hydrogen gas. pH of the medium increased from 7.3 to 7.5 due to removal of VFAs from the medium. The ORP of the fermentation medium decreased from -150 mV to -370 mV.

Figure 5.28 depicts variation of SHPR with light intensity, indicating a limitation by the availability of light for light intensities below 5 klux (I_{max}) and light inhibition above 5 klux. The data were correlated with the equations mentioned in section 4.1.3.2 and the coefficients were determined as following,

For $I \le I_m$, $\quad R = R_m \dfrac{I}{I_m}$ Eqn 5.2

where $R_m = 8.23$ mL g^{-1} biomass h^{-1} and $I_m = 5$ klux

or $R = k_s I = 1.646 I$, where $k_s = R_m/I_m$ is the light stimulation constant

For $I > I_m$, $\quad R = a - k_i I = 15.31 - 1.407 I$, $\quad (R^2 = 0.99)$

or $\quad \dfrac{R}{R_m} = a - b \dfrac{I}{I_m} = 1.853 - 0.853 \dfrac{I}{I_m}$ Eqn 5.3

where k_i (1.407) is the light inhibition constant.

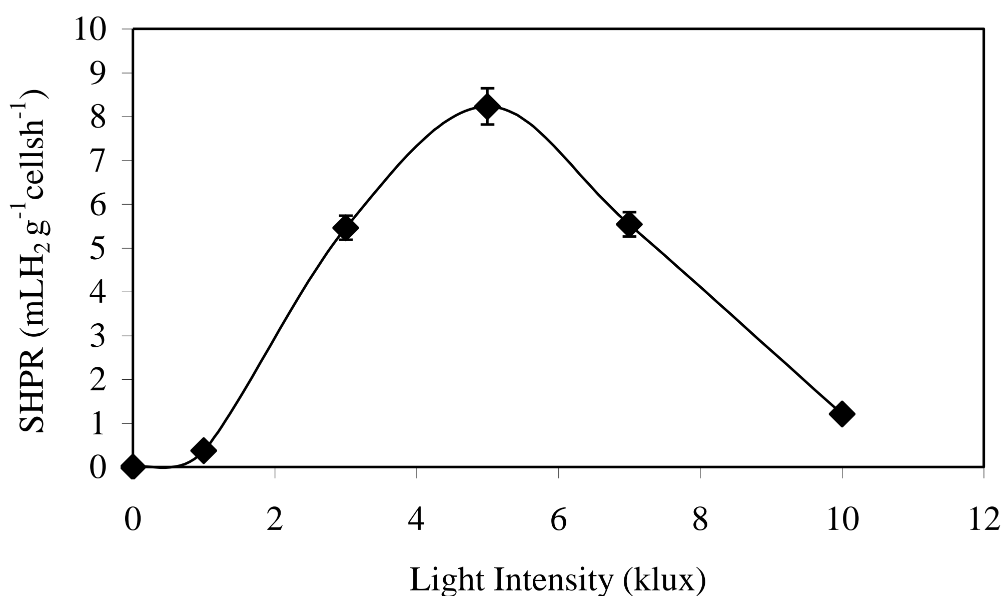


Figure 5.28 Variation of specific hydrogen production rate (SHPR) with light intensity in the light fermentation using *Rhodobacter sphaeroides*-RV and halogen lamp.

The results of this study are compared with the literature reports on light fermentation of dark fermentation effluents in Table 5.28. Since different bacterial strains, substrates, experimental conditions, light sources and operating modes were used in different studies listed in Table 5.28 it is difficult to make one to one

comparison. Total hydrogen yield (4.55 mol H_2 mol^{-1} glucose) obtained in this study (halogen lamp at 5 klux intensity) is higher than some of the reported studies (Chen et al., 2007; Obeid et al., 2008; Tao et al., 2007b;) and is lower than some (Lee et al., 2002; Yokoi et al., 2001; Yokoi et al., 2002). Higher hydrogen yield reported in some studies (Yokoi et al., 2001; Yokoi et al., 2002) may be due to over estimation of hydrogen production and/or under estimation of substrate utilization. None of the reported studies compared different light sources and identified the most suitable one. This study revealed that halogen lamp and 5 klux are the most suitable light source and intensity yielding high hydrogen yields and formation rates.

Table 5.28. Comparison of total hydrogen yields of light fermentations from different dark fermentation effluents.

Inoculum Culture and operation mode	**Substrate**	**Light source**	**Light intensity**	**H_2 Yield: mol H_2 mol^{-1} glucose**	**Reference**
Rhodopseudomonas palustris WP3-5- Continuous	DFE of hydrolyzed starch feedstock	Halogen lamp	95 Wm^{-2}	3.09	Tao et al. (2007b)
Rhodobacter sp M-19 Repeated Batch	DFE of waste starch	Incandescent lamp	5 klux	7.2	Yokoi et al. (2002)
Rhodobacter sp M-19 Repeated batch	DFE of sweet potato starch	Incandescent lamp	5 klux	7	Yokoi et al. (2001)
Rhodobacter sp M-19 batch	DFE of starch	Incandescent lamp	5 klux	5.5	Lee et al. (2002)
Mixture of three different *Rhodobacter* sp-Batch	DFE of wheat powder solution	Fluorescent lamp	5.5 klux	3.85	Obeid et al. (2008)
Rhodobacter sp-RV- Batch	DFE of wheat powder solution	Halogen lamp	5 klux	4.55	Our result

DFE: Dark Fermentation Effluent

5.1.3 Batch Experiments of Combined Fermentation

5.1.3.1 Effects of Dark to Light Biomass Ratio (D/L)

In order to determine the most suitable dark/light biomass ratio (D/L) for hydrogen production by dark-light combined fermentation of wheat powder solution, a set of batch experiments were performed at different D/L ratios between 1/2 and 1/10. Figure 5.29 depicts variation of cumulative hydrogen with time for different D/L ratios and also for dark and light fermentations alone. Cumulative hydrogen formation reached a steady level after 200 h incubation period for most of the cultures. However, fermentation was completed in 130 h when D/L ratio was 1/10 and in 150 h when the D/L ratio was 1/7. Hydrogen production by the cultures with D/L ratio of 1/2 and 1/4 was rather slow reaching steady levels in 180 h. Apparently, inclusion of high concentration of light fermentation bacteria (a mixture of *Rhodobacter* sp) into the fermentation media improved the rate of hydrogen formation and reduced the fermentation time by fermenting VFAs produced by dark fermentation bacteria.

The highest CHF (98 mL) was obtained with the dark or light fermentations alone where the light fermentation was considerably faster than the dark fermentation. The reason for this is the fact that dark fermentation bacteria can only convert glucose to VFA, H_2 and CO_2 with no further fermentation of VFAs to H_2 and CO_2. On the other hand, light fermentation bacteria are capable of fermenting glucose to VFAs, H_2 and CO_2 and also fermenting the VFAs further to H_2 and CO_2 resulting in faster and much higher hydrogen formation. In dark-light combined fermentation experiments, the highest CHF (76 mL) was obtained with the D/L ratio of 1/7. Further reductions and increases in the D/L ratio resulted in lower CHF. At high D/L ratios, the amount of light fermentation bacteria was not sufficient to convert VFAs to H_2 and CO_2 as fast as VFAs were produced by the dark fermentation bacteria (light fermentation limitation). At very low D/L ratios such as 1/10 the amount of dark bacteria was not sufficient to produce VFAs fast enough for effective fermentation by the light bacteria (dark fermentation limitation). Probably, the VFA formation rate of the dark bacteria was 7 times higher than VFA fermentation rate by the light bacteria yielding an optimum D/L ratio of 1/7 for balanced dark and light fermentations. Therefore,

using Light and dark fermentation bacteria in combination at a D/L ratio of 1/7 improved the rate and the yield of hydrogen formation.

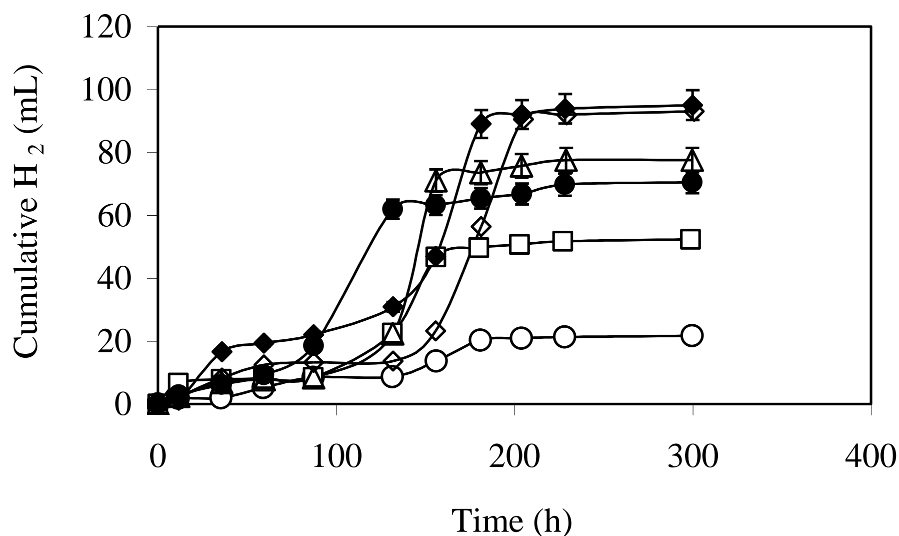


Figure 5.29 Variation of cumulative hydrogen with time in combined fermentation for different D/L biomass ratios . (○) 1/2 , (□) 1/4, (Δ) 1/7 , (●) 1/10, (◊) Dark, (♦) Light

Cumulative hydrogen data presented in Figure 5.29 were correlated with the Gompertz equation and the constants were determined by regression analysis as explained in section 0. The Gompertz equation constants for the combined dark-light fermentations are presented in Table 5.29 for different D/L ratios. The highest hydrogen production (P = 98 mL) was realized with either the light or dark fermentation. However, the rate of hydrogen formation was low for the dark (1.36 mL h^{-1}) and light (1.63 mL h^{-1}) fermentations. In combined dark-light fermentations, the highest rate (3.32 mL h^{-1}) and extent (76 mL) of hydrogen formation was obtained with the D/L ratio of 1/7 for the reasons explained above.

Table 5.29 Gompertz equation constants for different D/L biomass ratios.

D/L biomass ratio	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
1/2	25.31	0.12	26.65	0.95
1/4	61.98	0.30	26.22	0.92
1/7	76.23	3.23	124.76	0.98
1/10	69.43	1	64.68	0.98
Dark	97.64	1.46	138.10	0.95
Light	98.74	1.32	111.44	0.91

Specific hydrogen production rates (SHPR) and hydrogen yields (HY) in combined dark-light fermentations are compared for different D/L ratios in Table 5.30. The highest hydrogen yield (1.16 mol H_2 mol^{-1} glucose = 176.65 mL H_2 g^{-1} starch) and the SHPR (12.17 mL H_2 . g^{-1} cells. H^{-1}) were obtained with a D/L ratio of 1/7. Hydrogen yields obtained with only light and dark fermentations were slightly higher than the combined fermentation. However, combined fermentation with optimum D/L ratio of 1/7 resulted in considerably higher SHPR (12.17 mL H_2 . g^{-1} cells. H^{-1}) as compared to the dark and light fermentations alone (5 mLH_2 g^{-1} cellsh^{-1}) indicating advantages of using combined dark-light fermentation to reduce the fermentation time and to improve the hydrogen productivity.

Table 5.30 Hydrogen formation rates and the yields in combined fermentation at different D/L ratios.

D/L biomass ratio	**Yield (mol H_2 mol^{-1} glucose)**	**Yield (mL H_2 g^{-1} starch)**	**SHPR (mLH_2 g^{-1} cells h^{-1})**
1/2	0.27	40.96	0.45
1/4	0.80	113.68	1.14
1/7	1.16	176.55	12.17
1/10	0.91	138.87	3.76
Dark	1.31	200.47	5.16
Light	1.24	189	4.96

Table 5.31 summarizes the initial and final TVFA, NH_4-N and starch concentrations in combined fermentation at different D/L ratios. Initial TVFA concentrations were between 100-200 mgL^{-1} carried by the ft he ms cultures. Final TVFA concentrations varied between 86 and 521 mgL^{-1} which were all below inhibitory level of 2500 mgL^{-1} for the light fermentation organisms. The highest final TVFA (521 mgL^{-1}) was obtained with the dark fermentation alone indicating inability of the dark fermentation bacteria in fermenting VFAs to H_2 and CO_2. Final

TVFA concentrations decreased by inclusion of the light fermentation bacteria into the medium due to effective breakdown of TVFAs to H_2 and CO_2 by the photo-fermentative bacteria. The lowest final TVFA (86 mgL^{-1}) was obtained with the D/L ratio of 1/7 indicating effective fermentation of VFAs by the light bacteria to produce H_2 gas. The NH_4-N levels (nearly 10 mgL^{-1}) were all below the inhibitory level (50 mg NH_4-N L^{-1}) for the *Rhodobacter* sp. Starch hydrolysis and utilization was above 80% in all experiments indicating effective hydrolysis of starch by the dark fermentation bacteria. Light fermentation bacteria (*Rhodobacter sp.*) are known to be incapable of hydrolyzing starch. However, the bacteria from the natural flora of wheat powder or contaminant bacteria from other sources were probably effective in starch hydrolysis in light fermentation alone. In fact in many of our dark fermentation experiments, the un-inoculated serum bottles (control experiments) yielded high levels of hydrogen formation due to the activity of the natural flora of the wheat powder or contaminant bacteria from other sources. In control experiments nearly 40 mL H_2 was produced within 200 h due to the activity of the contaminant bacteria since the experiments were performed under non-aseptic conditions. With the highest TVFA conversion to hydrogen, the combined fermentation with the D/L ratio of 1/7 resulted in the highest rate and the yield of hydrogen formation.

Table 5.31 Initial and final concentrations of TVFA, NH_4-N and starch for the combined fermentation at different D/L ratios.

D/L biomass ratio (g g^{-1})	**$TVFA_o$ (mgL^{-1})**	**$TVFA_F$ (mgL^{-1})**	**NH_4-N_o (mgL^{-1})**	**NH_4-N_F (mgL^{-1})**	**$Starch_o$ (mgL^{-1})**	**$Starch_F$ (mgL^{-1})**
1/2	165	95	10.40	5.60	4782	795
1/4	129	92	10.40	7.20	4029	793
1/7	151	86	11.20	5.60	4115	807
1/10	110	103	10.40	3.20	4636	814
Dark	207	521	4	4	4224	730
Light	101	163	12	5.60	4664	882

Figure 5.30 depicts variation of starch, glucose, VFA and cumulative hydrogen with time for the combined fermentation at the D/L ratio of 1/7. Starch hydrolysis to sugar took place within the first 50 h where nearly 80% of starch was hydrolyzed. Sugar concentration sharply decreased to nearly 0.1 gL^{-1} and remained at that level

throughout the fermentation period. TVFA was the intermediate in conversion of starch to hydrogen which increased to nearly 1.4 gL^{-1} within the first 50 h due to VFA formation by dark and light fermentation of glucose. TVFA concentration decreased in the later stages of fermentation due to fermentation of VFAs to H_2 and CO_2 by the photo-fermentative organisms. Two stages were observed in hydrogen production. In the first stage (within the first 80 h), hydrogen was mainly produced using glucose generated by the partial hydrolysis of wheat starch during boiling. Since the initial sugar content was low, very little hydrogen was produced during this stage. In the second stage, hydrogen was produced by using glucose generated from the enzymatic hydrolysis of starch present in the medium which was considerable.

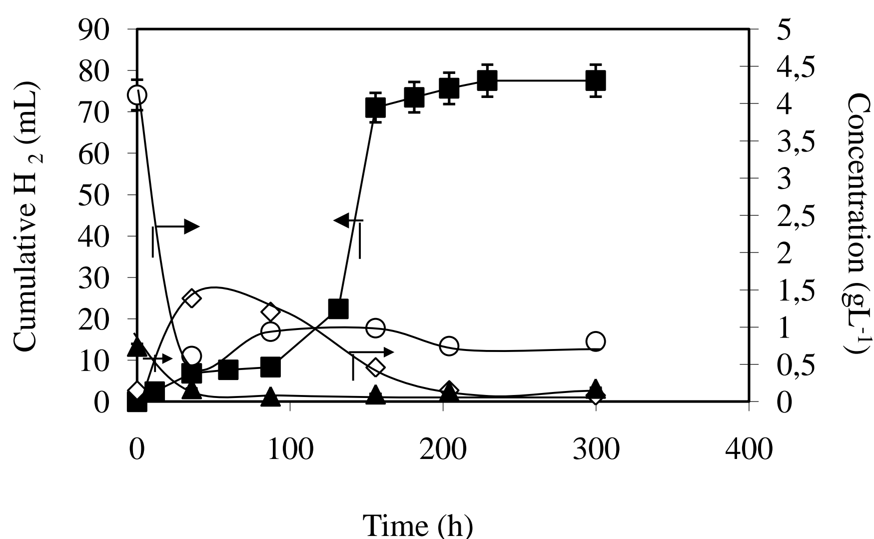
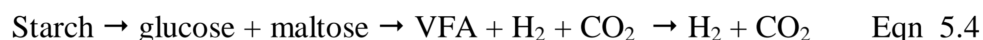


Figure 5.30 Variation of (■) Cumulative hydrogen (mL), (◊) Total volatile fatty acid (gL^{-1}), (▲) glucose (gL^{-1}) and (○) Starch (gL^{-1}) concentrations with time for the combined fermentation at the D/L ratio of 1/7.

Conversion of wheat starch to hydrogen gas by dark and light fermentations can be described as follows:



$$\text{Starch} \rightarrow \text{glucose} + \text{maltose} \rightarrow \text{VFA} + H_2 + CO_2 \rightarrow H_2 + CO_2 \qquad \text{Eqn 5.4}$$

The first reaction is the enzymatic hydrolysis of wheat starch to glucose and maltose by the dark fermentation bacteria. Light fermentation bacteria do not have

the capability of hydrolyzing the starch. The second reaction is the fermentation of sugar to VFA, H_2 and CO_2 by both the dark and light fermentation bacteria. The third step is the fermentation of VFAs to H_2 and CO_2 by the light fermentation bacteria (*Rhodobacter* sp). In the absence of light fermentation bacteria, the rate and the extent of hydrogen formation would be low, since hydrogen formation by the third reaction would not be realized. A combination of the dark and light fermentation bacteria can convert starch all the way to H_2 and CO_2 using all three reactions. However, the ratio of the dark to light fermentation bacteria must be right in order to avoid accumulation of VFAs in the medium. In other words, VFA formation and conversion must be balanced. That is why the D/L ratio of 1/7 resulted in the highest hydrogen formation rate and the yield. When only light fermentation bacteria were used, the bacteria present in the natural flora of the wheat powder or some contaminant bacteria from other sources helped hydrolysis of starch to sugar. Later reactions were carried out by the light fermentation bacteria producing high levels of hydrogen.

Very few studies were reported in literature on combined dark and light fermentations utilizing pure cultures of *Clostridium* and *Rhodobacter* species (Asada et al., 2006; Yokoi et al., 1998). Asada et al. (2006) used immobilized cultures of *Lactobacillus* and *Rhodobacter sphaeroides* RV in agar gels for hydrogen production from glucose solution and reported a maximum hydrogen yield of 7.1 mol H_2 mol^{-1} glucose. Yokoi et al. (1998) used a co-culture of *Clostridium butyricum* and *Rhodobacter sp*. for hydrogen production from starch and reported a hydrogen yield of 6.6 moles H_2 mol^{-1} glucose in a fed-batch culture. Fang et al. (2006) used *C. butyricum* and *R. sphaeroides* for combined fermentation of glucose to hydrogen and reported an optimum *Rhodobacter*/*Clostridium* (L/D) ratio of 5.9/1 yielding maximum CHF which is comparable with our results. Hydrogen yield in that study was calculated to be nearly 0.9 moles H_2 mol^{-1} glucose. The reported combined fermentation studies were all conducted under different conditions using immobilized or suspended pure cultures. For this reason it is difficult to compare the results with our study. Required fermentation times are all comparable (150-200 h). The higher hydrogen yields reported by Yokoi et al. (1998) and Asada et al. (2006) may be due to use of pure cultures at high concentrations in nutritionally rich

synthetic medium. The use of heat treated anaerobic sludge along with a mixed culture of *Rhodobacter* species in our study may have caused some adverse interactions among the cultures reducing the hydrogen yield. However, our results are in agreement with the study of Fang et al. (2006) in terms of the optimum D/L ratio (5.9/1 versus 7/1 in our study). The hydrogen yields obtained in our study (1.2 moles H_2 mol^{-1} glucose) is higher than that of the Fang et al. (2006).

5.1.3.2 Effects of Initial Substrate and Biomass Concentrations

5.1.3.2.1 Effects of Initial Wheat Powder Concentration. Time course of variations of cumulative hydrogen formation (CHF) for different initial wheat powder concentrations are depicted in Figure 5.31 for the combined fermentation. For low wheat powder concentrations (< 10 gL^{-1}) fermentation was completed within 50 h while nearly 100 h was required for complete fermentation for wheat powder concentrations above 10 gL^{-1}. Combined dark-light fermentation was much faster than dark and light fermentations alone which require more than 200 h for completion. Cumulative hydrogen formation (CHF) gradually increased with increasing initial wheat powder concentration. The highest CHF (135.5 mL) was obtained with the WP = 20 gL^{-1} and the lowest (18.2 mL) was with the WP = 2.5 gL^{-1} as expected.

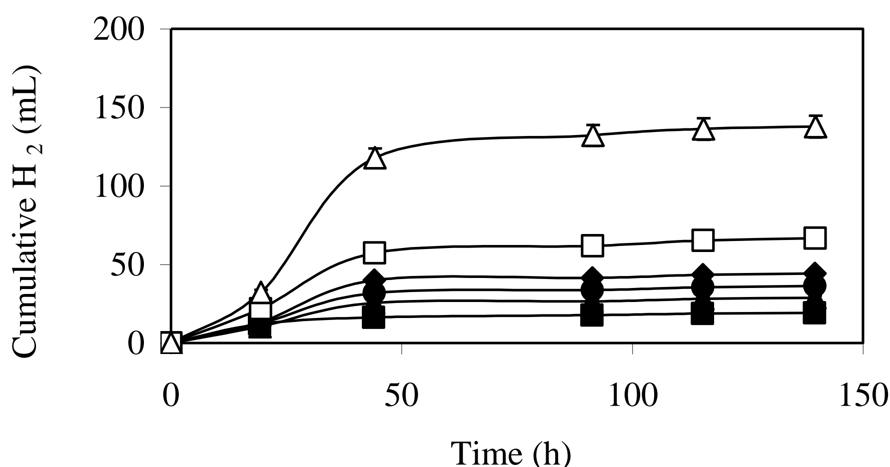


Figure 5.31 Variation of cumulative hydrogen formation with time in combined fermentation for different initial WP concentrations (g L^{-1}). (■) 2.5, (▲) 5, (●) 7.5, (♦)10, (□) 15, (Δ) 20. D/L = 1/10, X_T = 0.411 gL^{-1}.

Figure 5.32 depicts time course of variations of cumulative hydrogen, starch, total sugar, TVFA and NH_4-N concentrations in combined fermentation at WP = 20 gL^{-1}. CHF reached the higest level of 135.5 mL within 90 h. Nearly 90% of the initial starch was hydrolyzed and utilized within 120 h yielding a final starch content of nearly 2 gL^{-1}. The results showed fast and effective hydrolysis of starch for biohydrogen production in combined fermentation. Glucose concentration decreased from an initial level of 4 gL^{-1} (carried by the boiled and partially hydrolyzed starch) to less than 1 gL^{-1} throughout the fermentation period. The results indicated that once the wheat starch was hydrolyzed by the bacteria, the produced sugar was readily fermented to VFA, CO_2 and H_2. Part of the VFAs produced from dark fermentation was used by the light fermentation bacteria to produce CO_2 and H_2. However, production rate of VFA by dark fermentation was higher than the fermentation rate of VFA by the photo-fermentative bacteria resulting in VFA accumulation in the medium. TVFA concentration increased to nearly 7 gL^{-1} at the end of 120 h. With the initial and final starch concentrations of 16 gL^{-1} and 2 gL^{-1}, respectively, about 9.5 gL^{-1} TVFA formation would be expected by dark fermentation by assuming 60% conversion of glucose to VFA. The difference in theoretical and experimental VFA concentrations is due to fermentation of VFA to hydrogen and CO_2 by the light fermentation bacteria. NH_4-N concentration increased all the way to 120 mgL^{-1} probably due to NH_4-N release from disintegrated cells. The final TVFA and NH_4-N concentrations were above the inhibitory levels (which is 2.5 gL^{-1} for TVFA and 50 mgL^{-1} for NH_4-N) for the light fermentation bacteria (*Rhodobacter sp*) limiting conversion of TVFA to H_2 by the light fermentation. The initial wheat powder concentration should be less than 10 gL^{-1} in order to avoid inhibitions caused by high VFA ad NH_4-N concentrations and to improve hydrogen formation rate and the yield.

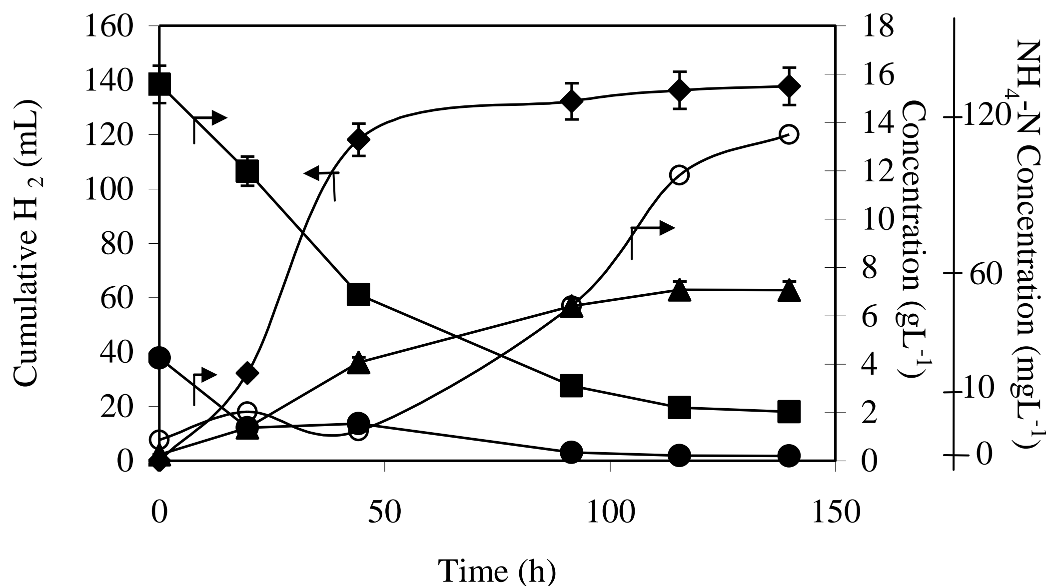


Figure 5.32 Variation of (♦) Cumulative hydrogen, (■) Starch, (▲) TVFA , (●) Glucose and (o) NH_4-N concentrations with time for the combined fermentation with 20g L^{-1} initial WP concentration.

Cumulative hydrogen formation data presented in Figure 5.32 were correlated with the Gompertz equation and the constants were determined by regression analysis according to section 04.1.2. The Gompertz equation constants for the combined dark-light fermentation at different WP concentrations are presented in Table 5.32. Maximum hydrogen formation potential (P, mL) increased with increasing wheat powder concentration and reached the maximum level (135.5 mL) at the WP =20 gL^{-1}. Maximum rate of hydrogen formation (Rm, mL h^{-1}) was almost constant for the wheat powder concentrations between 2.5 and 7.5 gL^{-1} which increased with increasing wheat powder concentration above 10 gL^{-1}. The maximum rate (3.44 mL h^{-1}) was obtained with 20 gL^{-1} wheat powder concentration. The Lag time (λ, h) also increased with increasing wheat powder concentration indicating requirement for longer adaptation periods at high substrate concentrations.

Table 5.32 Gompertz equation constants for different initial WP concentrations in combined fermentation

WP concentration (gL^{-1})	P (mL)	R_m (mL h^{-1})	λ (h)	R^2
2.5	18.18	0.17	15.80	0.98
5	27.24	0.61	17.20	0.99
7.5	35.24	0.77	17.00	0.99
10	43.04	1.08	14.31	0.99
15	64.75	1.44	18.96	0.99
20	135.47	3.44	13.52	0.99

Table 5.33 summarizes specific hydrogen production rates (SHPR) and hydrogen yields (HY) obtained at different wheat powder concentrations in combined dark-light fermentation. The highest hydrogen yield (HY = 0.42 mol H_2 mol^{-1} glucose or 63.9 mL H_2 g^{-1} starch) was obtained at the lowest wheat powder concentration of 2.5 gL^{-1}. This is due to low levels of VFA formation from dark fermentation and therefore, absence of VFA inhibition on the light fermentation bacteria. Initial wheat powder of 5 gL^{-1} yielded similar results. However, further increases in the initial wheat powder concentration above 5 gL^{-1} resulted in lower hydrogen yields due to formation of inhibitory levels of VFAs. The only exception for this trend was 20 gL^{-1} WP concentration where high hydrogen yields were obtained probably due to high cumulative hydrogen formation by dark fermentation bacteria. SHPRs were calculated by dividing the hydrogen formation rate (Rm) by the total amount of initial bacteria (SHPR = Rm / Vo Xo where Vo and Xo are the initial fermentation volume and biomass concentration, respectively). Since the initial amount of biomass was constant in all experiments, SHPR was determined by the hydrogen formation rate.

Table 5.33 Hydrogen yields and specific production rates for different initial WP concentrations in combined fermentation

WP Concentration (gL^{-1})	Yield (molH_2 mol^{-1}glucose)	Yield (mL H_2 g^{-1} starch)	SHPR (mLH_2 g^{-1} $cells^{-1}$)
2.5	0.42	63.90	2.31
5.0	0.40	61.27	8.32
7.5	0.30	46.59	10.60
10.0	0.25	38.65	14.71
15.0	0.25	37.96	19.62
20.0	0.37	55.52	46.87

The initial and final starch (substrate) and TVFA concentrations are summarized in Table 5.34. Starch utilization at low starch concentrations below 6 gL^{-1} were lower than 80% which increased with increasing starch concentration and reached 87% for the initial starch concentration of 20 gL^{-1}. High extent of starch hydrolysis yielded high sugar concentrations and therefore, high VFA and cumulative hydrogen formations. VFAs were formed by dark and light fermentation of glucose produced from starch hydrolysis. The initial VFA concentrations were carried by the inoculums of the microorganisms. The final VFAs increased steadily with increasing starch concentrations indicating effective conversion of glucose to VFAs by dark and light fermentation bacteria. However, the VFA concentrations above 2.5 gL^{-1} is known to be inhibitory for the light fermentation bacteria. The reason for low hydrogen yields at high initial starch concentrations above 10 gL^{-1} is probably formation of inhibitory levels of VFAs (> 3 gL^{-1}). Therefore, combined dark-light fermentations should be operated with the initial WP concentrations below 10 gL^{-1} or VFAs should be simultaneously removed from the media in order to avoid VFA inhibition.

Table 5.34 Initial and final TVFA and starch concentrations for different initial WP concentration in combined fermentation

WP Concentration (gL^{-1})	**$Starch_o$ (mgL^{-1})**	**$Starch_F$ (mgL^{-1})**	**$TVFA_o$ (mgL^{-1})**	**$TVFA_F$ (mgL^{-1})**
2.5	2193	511	83	439
5	3767	1129	166	1300
7.5	5846	1467	129	2150
10	8183	1782	157	3370
15	12048	2190	212	4870
20	15570	2029	246	7060

5.1.3.2.2 Effects of Initial Cell Concentration. In this set of experiments, wheat powder concentration was constant at 5 gL^{-1}, and the initial cell concentration was varied between 0.5 and 5 gL^{-1} with a constant D/L ratio of 1/7. Figure 5.33 depicts variation of cumulative hydrogen formation (CHF) with time for different initial cell concentrations. The highest CHF (118.4 mL) was obtained with an initial cell concentration of 1.1 gL^{-1} yielding an optimum biomass/ substrate ratio of 0.22 g

biomass/ g WP. At low initial cell concentrations (< 1.1 gL^{-1}), CHF increased with the cell concentration and reached the highest level at Xo = 1.1 gL^{-1} indicating Limitations by active cell concentration. Further increases in initial cell concentration above 1.1 gL^{-1} reduced the CHF due to limitations by the low substrate concentration. The lowest CHF (24.3 mL) was obtained with the highest initial biomass concentration of 5 gL^{-1}. Cumulative hydrogen produced with the control experiment (41 mL) was due to bacteria naturally present in the ground wheat or bacteria contaminated the medium since the experiments were performed under non-sterile conditions.

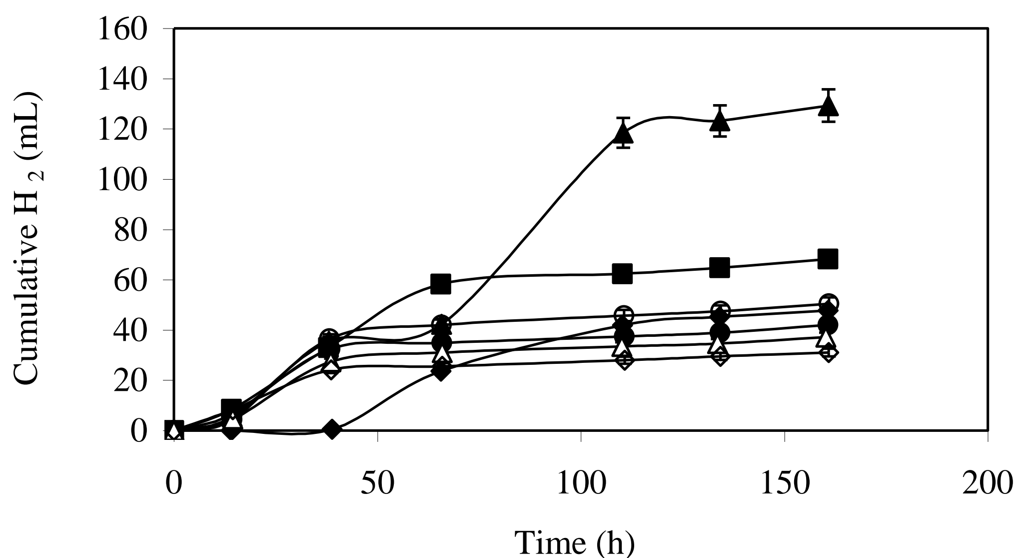


Figure 5.33 Variation of cumulative hydrogen formation with time in combined fermentation for different initial biomass concentration (g L^{-1}) (♦) Control, (■) 0.5, (▲) 1.1 , (○) 1.5, (●) 2.5 , (Δ) 3.5, (◊) 5 . WP = 5 g L^{-1}, D/L = 1/7.

Figure 5.34 depicts variations of starch, glucose, VFA , NH_4-N concentrations and cumulative hydrogen with time for the initial cell concentration of 1.1 gL^{-1}. Starch concentration decreased from 4gL^{-1} to 0.75 gL^{-1} within the first 100 h and then remained constant. Cumulative hydrogen formation showed an increase in two phases where the available sugar was fermented in the first phase and the hydrolyzed sugar in the second phase. A total of 118 mL H_2 was produced within 120 h. TVFA steadily increased within the first 100 h and then remained almost constant at 1520

mgL^{-1} for the rest of the fermentation period. Slight decrease in VFA at the end of the fermentation was due to conversion of VFAs to CO_2 and H_2 by the *Rhodobacter* sp. Glucose concentration increased slightly within the first 20 h as a result of starch hydrolysis and then decreased steeply due to conversion to VFA, CO_2 and H_2 by dark and light fermentation. The rate limiting step in conversion of wheat starch to hydrogen was starch hydrolysis since the soluble glucose concentrations were below 0.1 gL^{-1} indicating readily fermentation of glucose upon formation. NH_4-N concentration decreased steeply from 43 mgL^{-1} to less than 5 mgL^{-1} within the first 70 h due to cell bio-synthesis. NH_4-N concentrations were always below the inhibitory level (50 mgL^{-1}) for the *Rhodobacter* sp.

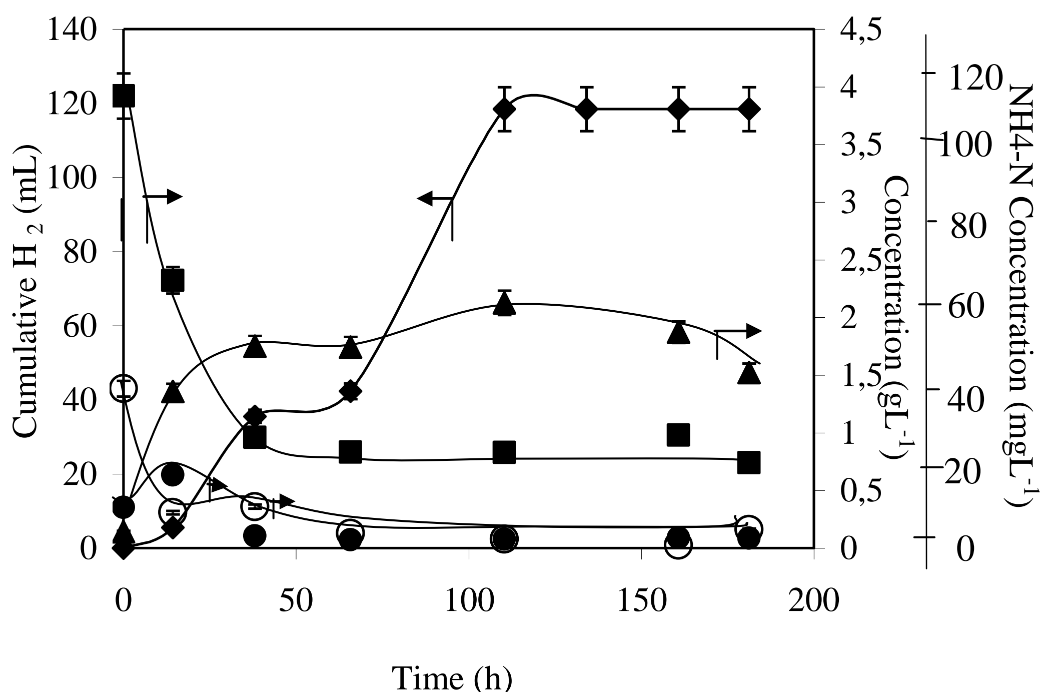


Figure 5.34 Variation of (♦) Cumulative hydrogen, (■) Starch, (▲) TVFA, (●) Glucose and (o) NH_4-N concentrations with time in combined fermentation for the initial cell concentration of 1.1 gL^{-1}. D/L = 1/7, WP = 5 gL^{-1}.

Cumulative hydrogen data presented in Figure 5.33 was correlated with the Gompertz equation and the constants are presented in Table 5.35 for different initial cell concentrations. Maximum hydrogen formation (P) increased with the initial cell concentration up to 1.1 gL^{-1} and then decreased with further increases yielding the

highest hydrogen formation (118 mL) with 1.1 gL^{-1} initial cell concentration (0.22 g cell/g WP). High initial cell concentrations yielded low hydrogen formation due to inavailibility of sufficient substrate. Maximum hydrogen formation rate (R_m) also increased with increasing initial cell concentration and reached the maximum level 1.71 mL h^{-1} at 1.1 gL^{-1} cell concentration with an optimum cell/substrate ratio of 0.22 g cell/gWP. Further increases in biomass concentration resulted in lower hydrogen formation rates due to lower initial So/Xo ratio. The lowest R_m value (0.65 mL h^{-1}) was obtained with the highest (5 g L^{-1}) cell concentration. Due to floc formation at high cell concentrations, interaction surface between the insoluble wheat powder particles and the cells decreased (inavailability of starch for the bacteria inside the flocs) yielding Low rates of starch hydrolysis and hydrogen formation. Duration of the lag phase (λ) varied between 9 and 20 h in experimental bottles with the highest lag phase of 55 h in the control experiment due to very low level of contamination biomass.

Table 5.35 Gompertz equation constants for different initial cell concentrations in combined fermentation.

Cell Concentration (gL^{-1})	P (mL)	R_m (mL h^{-1})	λ (h)	R^2
0 (Control)	41.93	0.58	38.80	0.99
0.5	59.05	0.97	10.64	0.99
1.1	118.4	1.71	65.80	0.96
1.5	42.12	1.35	12.85	0.99
2.5	33.46	1.08	9.08	0.99
3.5	28.77	0.94	11.26	0.99
5.0	24.30	0.65	14.35	0.99

Variations of hydrogen yields (HY) and specific hydrogen production rates (SHPR) with the initial cell concentration are summarized in Table 5.36. Again the highest hydrogen yield (1.03 moles H_2 $moles^{-1}$ glucose or 156.8 mL H_2 g^{-1} starch) was obtained with the initial cell concentration of 1.1 g L^{-1} yielding an optimum cell/ substrate ratio of 0.22 g cell/ g WP. At low initial cell concentrations (Xo < 1.1 gL^{-1}) the rate and the extent of hydrogen formation was limited by the low bio-catalyst (cell) concentration. Hydrogen formation was limited by the inavailability of the insoluble substrate due to bacterial floc formation at high initial cell concentrations. SHPR steadily decreased with the increasing initial cell concentration since both R_m

decreased and X_o increased with the increasing initial cell concentration. The highest SHPR (8.15 mL H_2 g^{-1} cell h^{-1}) was obtained with the lowest Xo (0.5 gL^{-1}) as expected. Since the important criteria in selecting the operating conditions are the hydrogen yield and the highest formation rate (R_m) the optimum cell concentration was determined to be 1.1 gL^{-1}.

Table 5.36 Hydrogen yields and specific production rates for different initial cell concentrations in combined fermentation

Cell Concentration (gL^{-1})	**Yield (mol H_2 mol^{-1} glucose)**	**Yield (mL H_2 g^{-1} starch)**	**SHPR (mLH_2 g^{-1} cells h^{-1})**
0 (Control)	0.32	49.08	-
0.5	0.48	74.62	8.15
1.1	1.03	156.82	6.54
1.5	0.33	51.62	3.78
2.5	0.28	43.59	1.83
3.5	0.27	42.20	1.13
5	0.22	33.82	0.55

Initial and final starch, TVFA and NH_4-N concentrations are summarized in Table 5.37 for the variable cell concentration experiments. Initial starch was constant at 4 ± 1gL^{-1} in all experiments which decreased to 0.6 ± 0.2 gL^{-1} for the experiments with the initial cell concentration below 2.5 gL^{-1}. However, the final starch concentration was above 1 gL^{-1} for the high initial cell concentrations of above 2.5 gL^{-1}. Percent starch utilization was above 80% for low cell concentrations (< 1.5 gL^{-1}) which decreased to 72% for the cell concentration of 5 g L^{-1}. Wheat powder is an insoluble substrate requiring direct contact between the substrate and the cell. At high cell concentrations (> 2.5 gL^{-1}) due to cell aggLomeration and floc formation the effective cell concentration in contact with the insoluble wheat particles decreased yielding Low degree of starch utilization. Initial NH_4-N concentrations were all below the inhibitory level (< 50 mgL^{-1}) for the *Rhodobacter* sp. The final NH_4-N concentrations indicated effective utilization of NH_4-N for bacterial growth which was not that extensive due to low levels of NH_4-N. Initial TVFAs present in the medium were probably carried by the ft he ms culture. Final TVFA concentrations were 1.5 ± 0.2 gL^{-1} for low cell concentrations(< 1.5 gL^{-1}) indicating effective dark fermentation of glucose derived from starch hydrolysis to VFAs, CO_2

and H_2. For the cell concentration of 1.1 gL^{-1}, the utilized starch (S_o- S_f) was nearly 3200 mgL^{-1}. Assuming nearly 60% of starch conversion to VFAs, one would expect nearly 1920 mgL^{-1} final VFA concentration. However, the actual final VFA was 1523 mgL^{-1} indicating nearly 400 mgL^{-1} VFA conversion to CO_2 and H_2 by the *Rhodobacter* sp. At high cell concentrations (> 2.5 gL^{-1}) due to cell aggLomeration reducing the insoluble substrate (WP)-cell contact area, starch hydrolysis and VFA formation were low yielding Low levels of hydrogen formation. Therefore, high cell concentrations are not recommended for insoluble wheat starch hydrolysis and fermentation for hydrogen formation. The optimum cell concentration yielding the highest level of starch hydrolysis, VFA and H_2 formation was 1.1 gL^{-1} for the initial wheat powder concentration of 5 gL^{-1} yielding an optimum cell/substrate ratio of 0.22 g cells / gWP.

Table 5.37 Initial and final TVFA, NH_4-N and starch concentrations for different initial cell concentrations in combined fermentation.

X_T (gL^{-1})	TVFAo (mgL^{-1})	$TVFA_F$ (mgL^{-1})	NH_4-N_o (mgL^{-1})	NH_4-N_F (mgL^{-1})	$Starch_o$ (mgL^{-1})	$Starch_F$ (mgL^{-1})
0 (Control)	97	1426	44	30	4046.59	449.77
0.5	8	1758	37	3	3921.74	624.19
1.1	142	1523	43	5	3921.74	740.48
1.5	94	1732	40	8	4058.34	651.74
2.5	154	596	39	10	3987.82	856.77
3.5	155	730	43	9	3904.11	1034.26
5.0	215	395	29	3	4149.41	1129.13

The results of this study are compared with literature reports on hydrogen gas production by combined dark-light fermentation in Table 5.38.

Table 5.38 Comparison of hydrogen yields and formation rates in combined dark-light fermentations.

Substrate	H_2 Yield ($mLH_2\ g^{-1}$starch)	H_2 Formation Rate ($mLH_2\ g^{-1}$cells h^{-1})	Reference
Glucose	7.1 $molH_2\ mol^{-1}$ glucose	not reported	Asada et al. (2006)
Starch	6.6 $molH_2\ mol^{-1}$ glucose	not reported	Yokoi et al. (1998)
Glucose	0.86 $molH_2\ mol^{-1}$ glucose	not reported	Fang et al. (2006)
Boiled wheat powder	157 $mLH_2\ g^{-1}$starch = 1.05 mol H_2 mol^{-1} glucose	47 $mLH_2\ g^{-1}$cells h^{-1}	Our result

5.1.3.3 Effects of Light source, Light Intensity and Lighting Regime

5.1.3.3.1 Effects of Light Source. Six batch combined fermentation experiments were performed with different light sources by using ground wheat starch as the substrate. The light intensity was constant for all light sources at 270 Wm^{-2}. Figure 5.35 depicts variation of cumulative hydrogen formation (CHF) with time for different light sources. The highest CHF (178 mL) was obtained with halogen lamp and the lowest was with fluorescent light (106.4 mL). Tungsten light yielded higher CHF (153.5 mL) than the tungsten + IR lights (127 mL). IR light alone yielded 131 mL CHF. Illumination with sunlight yielded 125.5 mL H_2 which was comparable with that obtained with IR light. On the basis of the CHF results, the halogen lamp was the most suitable light source among the others tested.

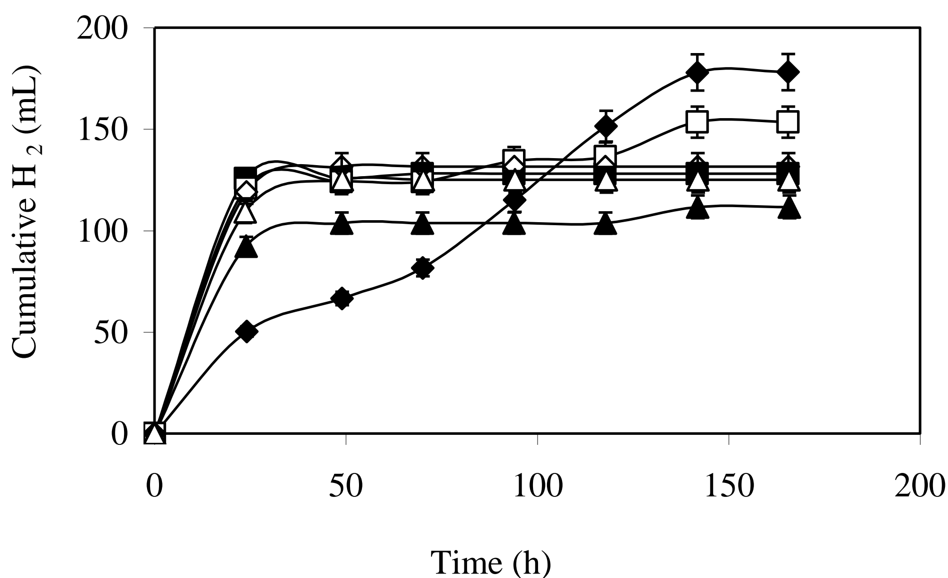


Figure 5.35 Variation of cumulative hydrogen formation with time for different light sources in combined fermentation (■) Tung + IR, (□) Tung, (◊) IR, (▲)Fluorescent, (♦) Halogen, (Δ) Sunlight.

Data from Figure 5.35 was correlated with the Gompertz equation according to section 0. Table 5.39 presents the Gompertz equation coefficients for different light sources. Maximum potential of hydrogen formation (P= 178 mL) was obtained with

halogen lamp with the lowest hydrogen formation rate (R_m = 1.34 mL h^{-1}). Probably halogen lamp was somewhat unusual to the dark fermentation bacteria and required some time for adaptation. The highest rate of hydrogen formation was obtained with the Tung + IR light (5.28 mL h^{-1}) although Tung (5.06 mL) and IR lights (4.96 mL) alone yielded comparable hydrogen formation rates. Apparently, IR light provided heat and high wavelength light source to improve the rate of hydrogen formation. Hydrogen gas formation rate obtained with sun-light (4.61 mL h^{-1}) was higher than that obtained with the fluorescent light (3.85 mL h^{-1}). The halogen lamp resulted in the lowest rate but the highest CHF. The lag phase varied between 3.7 and 5.2 h and the lowest lag was obtained with the halogen lamp (0.73 h). Sunlight resulted in the longest lag period (5.2 h) probably due to varying Light intensities during the day.

Table 5.39 Gompertz equation constants for different light sources in combined fermentation ($Starch_o$ = 4260 mgL^{-1}, NH_4-N_o = 6.5 mgL^{-1}, Light intensity = 270 Wm^{-2}).

Light Source	P (mL)	R_m (mL h^{-1})	λ (h)	R^2
Tung	153.49	5.06	3.73	0.94
Tung & IR	127.72	5.28	4.31	0.99
IR	131.60	4.96	4.62	1.00
Fluorescent	106.41	3.85	4.69	0.99
Halogen	178.14	1.34	0.73	0.97
Sun	125.50	4.61	5.21	1.00

Table 5.40 summarizes hydrogen yields and specific hydrogen production rates (SHPR) for different light sources in combined fermentation. The hydrogen yields varied between 138 and 218 mL H_2 g^{-1} starch. The highest yield was obtained with halogen lamp (218 mL H_2 g^{-1} starch) and the lowest with fluorescent light (138 mL H_2 g^{-1} starch). Tungsten light resulted in a hydrogen yield (189 mL H_2 g^{-1} starch) comparable to that of the halogen lamp. IR and Tung + IR lights yielded 162 and 157 mL H_2 g^{-1} starch, respectively. The lowest yield was obtained with fluorescent lamp (138 mL H_2 g^{-1} starch). Hydrogen yield obtained with sun-light (153 mL H_2 g^{-1} starch) was comparable to that obtained with Tung + IR lights. Theoretical hydrogen yield in combined dark-light fermentations is 12 moles H_2 mol^{-1} glucose if acetic acid is the only VFA produced by dark fermentation. The highest yield obtained with halogen lamp (1.45 moles H_2 mol^{-1} glucose) was well below the theoretical yield due to formation of a mixture of VFAs, and utilization of glucose for growth,

maintenance and other cellular activities. Besides, VFAs produced by dark fermentation were not completely converted to CO_2 and H_2 ($TVFA_f$ = 1.1 gL^{-1}) by the *Rhodobacter* sp. causing Low hydrogen yields. Most of the hydrogen was produced by dark and a lower fraction by light fermentation.

Specific hydrogen production rates (SHPR) based on the initial biomass concentration are also listed in Table 5.40. SHPRs varied between 13 and 51 mL H_2 g^{-1} biomass h^{-1} where the lowest SHPR was obtained with halogen (13 mL H_2 g^{-1} biomass h^{-1}) and the highest with tungsten +IR lamps (51 mL H_2 g^{-1} biomass h^{-1}). Hydrogen production with halogen lamp was rather slow probably due to inhibitory effects of halogen light on the dark fermentation bacteria. Tungsten and IR lights alone yielded 49 and 48 mL H_2 g^{-1} biomass h^{-1}, respectively which were comparable with the maximum SHPR obtained with the Tung + IR lights (51 mL H_2 g^{-1} biomass h^{-1}). SHPR obtained with the sunlight illumination (44.6 mL H_2 g^{-1} biomass h^{-1}) was comparable with that of the tungsten lamp. Apparently, most of the hydrogen was produced by dark fermentation and for this reason the difference in hydrogen yields obtained with different light sources (Tung, Tung + IR, IR, sunlight) was not significant.

Table 5.40 Hydrogen yields and SHPRs for different light sources in combined fermentation (Light intensity = 270 W m^{-2}).

Light Source	**$Starch_o$ (mgL^{-1})**	**$Starch_F$ (mgL^{-1})**	**Yield (mL H_2 g^{-1} starch)**	**Yield (molH_2 mol^{-1} glucose)**	**SHPR (mLH_2 g^{-1} biomass h^{-1})**
Tung	4260.70	196.10	188.79	1.26	48.94
Tung & IR	4260.70	188.75	157.32	1.05	51.06
IR	4260.70	212.47	162.67	1.08	47.97
Fluorescent	4260.70	214.46	138.01	0.92	37.23
Halogen	4260.70	168.75	217.77	1.45	13.00
Sun	4260.70	176.92	153.24	1.02	44.58

Variations of cumulative hydrogen (mL), TVFA (mgL^{-1}), glucose (gL^{-1}) and NH_4-N (mgL^{-1}) concentrations with time are depicted in Figure 5.36 for halogen lamp. TVFA concentration increased from an initial level of 0.5 gL^{-1} to nearly 1.8 gL^{-1} due to dark fermentation of glucose and then decreased to nearly 1.1 gL^{-1} due to light fermentation at the end of 170 h. Glucose concentration decreased from 4.2 gL^{-1} to less than 0.1 gL^{-1} within 50 h indicating fast conversion of glucose to VFAs and H_2

by dark fermentation. Hydrogen gas production showed a trend similar to diauxic growth. Dark fermentation took about 50 h and the light fermentation started after a short lag phase and lasted about 100 h. Initial NH_4-N concentration was 6.5 mgL^{-1} which decreased to less than 1 mg L^{-1} at the end of 150 h indicating utilization of NH_4-N for bacterial growth. pH of the medium decreased from 7.0 to 5.85 due to formation of VFAs in the medium and afterwards varied between 6.7-7.0. The ORP of the medium was around -125 ± 20 mV throughout the fermentation.

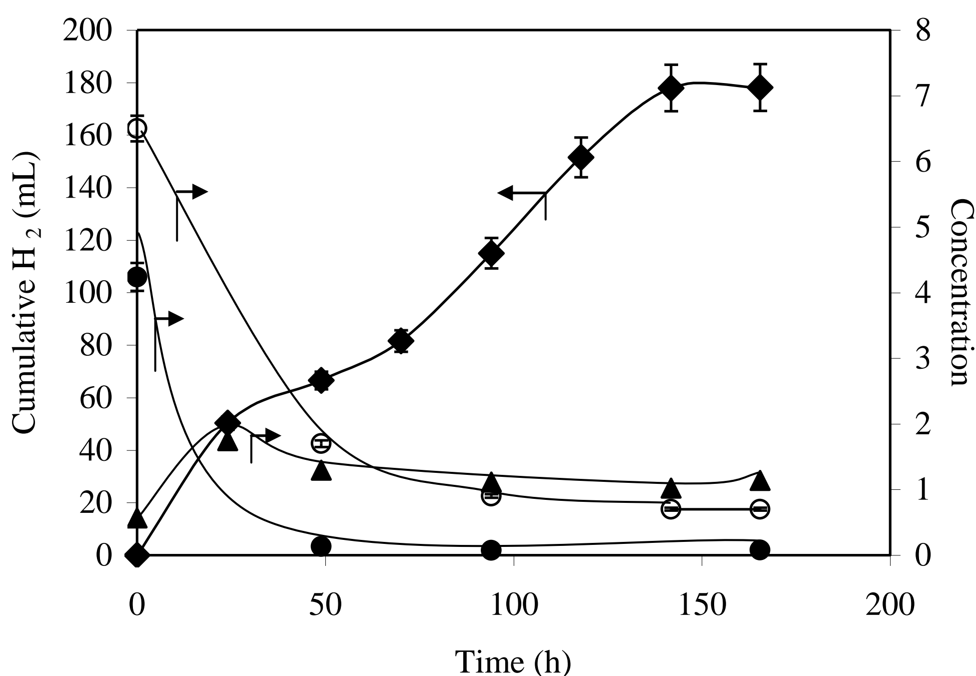


Figure 5.36 Variation of (♦) Cumulative hydrogen (mL), (▲) Total volatile fatty acid (gL^{-1}), (●)glucose (gL^{-1}) and (○) NH_4-N (mg L^{-1}) concentrations with time for combined fermentation with halogen lamp.

Since the highest CHF and hydrogen yields were obtained with halogen lamp, this light source was used in further experiments to determine the most suitable light intensity and lighting regime yielding the highest rate and extent of hydrogen gas production.

5.1.3.3.2 Effects of Light Intensity. Halogen lamp was used at five different light intensities (1-10 klux) in combined fermentations. An experiment with sun light was also used Figure 5.37 depicts the variation of CHF with time for different light intensities and for the sun light. CHF increased with increasing Light intensity and reached the maximum level (111 mL) at 10 klux (approx. 352 Wm^{-2}) light intensity. Apparently, availability of the light was the limiting factor in bio-hydrogen formation at all light intensities below 10 klux. The sunlight yielded 61 mL H_2 within 70 h which was comparable with the CHF obtained with 1 klux light intensity. The combined fermentation media was dark-brown colored preventing Light penetration through the media. Therefore, 10 klux light intensity on the surface of the bottles may be much lower inside the fermentation media. In light fermentation of the dark fermentation effluent, the optimum light intensity was found to be 5 klux above which light inhibition was observed. Combined fermentation required higher light intensities (> 5klux) due to light penetration problems to the dark-brown fermentation media.

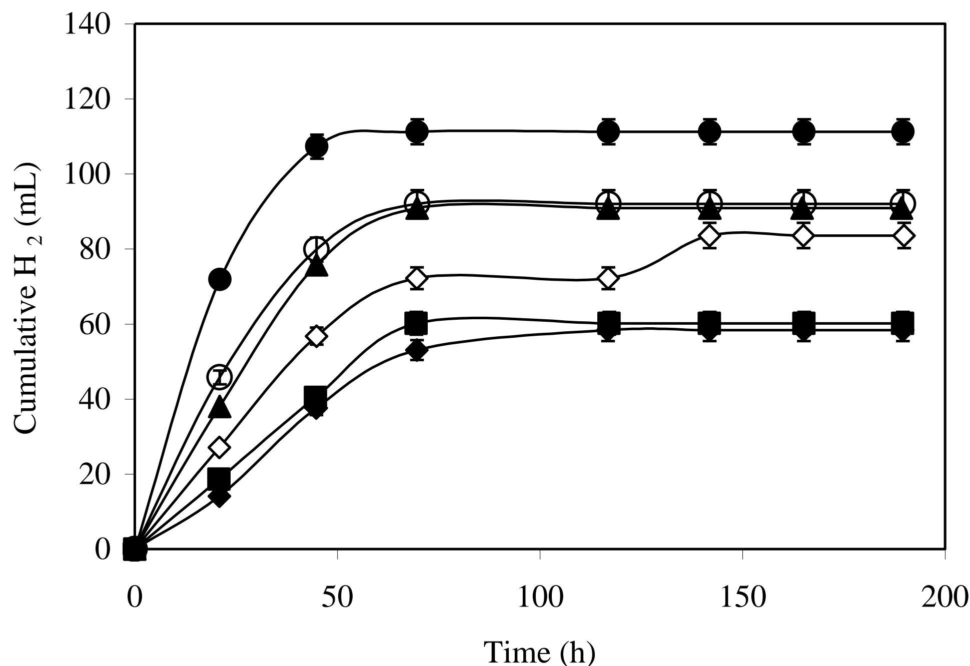


Figure 5.37 Variation of cumulative hydrogen formation with time for different light intensities in combined fermentation using halogen lamp. (♦) 1 klux, (◊) 3 klux, (▲) 5 klux, (o) 7 klux, (●) 10 klux, (■) Sun.

The data presented in Figure 5.37 was correlated with the Gompertz equation, the constants were determined by regression analysis and are listed in Table 5.41 for different light intensities. Similar to increases in CHF with the light intensity, the rate of hydrogen formation (R_m) also increased with the light intensity and reached the highest level (4.73 mL h^{-1}) at 10 klux. The lag phase varied between 3 and 8 h without any regular trend.

Table 5.41 Gompertz equation constants for different light intensities in the combined fermentations with the halogen lamp ($Starch_o$ = 4142 mg L^{-1}, NH_4-N_o = 7.66 mg L^{-1}).

Light Intensity (ft)	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
1	58.60	1.12	8.97	0.999
3	81.00	1.46	3.38	0.985
5	91.20	2.39	5.38	0.998
7	91.98	2.63	3.97	0.998
10	111.11	4.73	5.31	0.999
Sun light	60.82	1.24	6.96	0.992

The hydrogen yields and specific production rates (SHPR) are presented in Table 5.42 for different light intensities with halogen lamp. Utilization of starch was more than 95% for all light intensities ($Starch_o$ = 4140 mgL^{-1} , $Starch_f$ < 200 mgL^{-1}). Hydrogen yields increased from 73 mL H_2 g^{-1} starch to 139 mL H_2 g^{-1} starch when the light intensity increased from 1 klux to 10 klux. Illumination with sun-light yielded 75.7 mL H_2 g^{-1} starch which is somewhat lower than the reported yield in Table 5.40 (153 mL H_2 g^{-1} starch) due to variations in the intensity of the sun-light depending on the day. The hydrogen yield of 139 mL H_2 g^{-1} starch obtained with halogen lamp at 10 klux intensity is about 23% of the theoretical hydrogen yield of dark fermentation of starch (approx. 600 mL H_2 g^{-1} starch) when acetic acid is the only VFA produced. This is because of formation of a mixture of VFAs, utilization of starch for growth and maintenance and possible inhibition effects of light on dark fermentation. As shown in Figure 5.37, CHF was completed within 70 h where conversion of glucose to VFAs, CO_2 and H_2 took place by the anaerobic sludge and *Rhodobacter* sp. with no further hydrogen production by light fermentation of the VFAs. Apparently, light fermentation was not functional in this set of experiments probably due to high VFA, NH_4-N and other toxic metabolites which may have been

produced by the dark fermentation. However, *R. sphaeroides*-RV was probably active in production of hydrogen from glucose of hydrolyzed starch.

Table 5.42 Hydrogen yields and SHPR for different light intensities in combined fermentations with the halogen lamp ($Starch_o$ = 4142 mgL^{-1}, NH_4-N_o = 7.66 mgL^{-1}).

Light Intensity (klux)	$Starch_o$ (mgL^{-1})	$Starch_F$ (mgL^{-1})	Yield (mL H_2 g^{-1} starch)	Yield ($molH_2mol^{-1}$ glucose)	SHPR (mLH_2 g^{-1} biomass h^{-1})
1	4142	184	73.66	0.49	7.50
3	4142	171	105.22	0.70	9.76
5	4142	204	115.45	0.77	15.99
7	4142	169	115.75	0.77	17.60
10	4142	163	139.74	0.93	31.68
Sun Light	4142	168	75.70	0.51	8.32

Table 5.42 also summarizes SHPRs obtained with different light intensities. SHPRs increased from 7.5 mLH_2 g^{-1} biomass h^{-1} to 31.7 mLH_2 g^{-1} biomass h^{-1} when light intensity increased from 1 klux to 10 klux. Sun-light resulted in a SHPR of 8.32 mL H_2 g^{-1} biomass h^{-1} which is close to the SHPR obtained with 1 klux light intensity.

Variations of cumulative hydrogen (mL), TVFA (gL^{-1}), glucose (gL^{-1}) and NH4-N (mgL^{-1})concentrations with time are depicted in Figure 5.38 for combined fermentation with halogen lamp at 10 klux light intensity. TVFA produced by dark fermentation increased to 1.7 gL^{-1} within 50 h and remained constant for the rest of fermentation period indicating no photo-fermentation of VFAs for hydrogen production. Glucose concentration decreased from 4.1 gL^{-1} to less than 200 mgL^{-1} within 50 h indicating effective fermentation of glucose by the dark and light fermentation bacteria for production of VFA, CO2 and H2. A decrease in NH4-N concentration from 7.6 mgL^{-1} to nearly 1.0 mgL^{-1} at the end of fermentation indicated utilization of NH4-N for bacterial growth. pH of the medium decreased from 7.0 to 5.63 due to formation of VFAs in the medium and afterwards varied between 6.5-7. The ORP of the medium decreased from – 160 mV to – 443 mV at the end of the experiment.

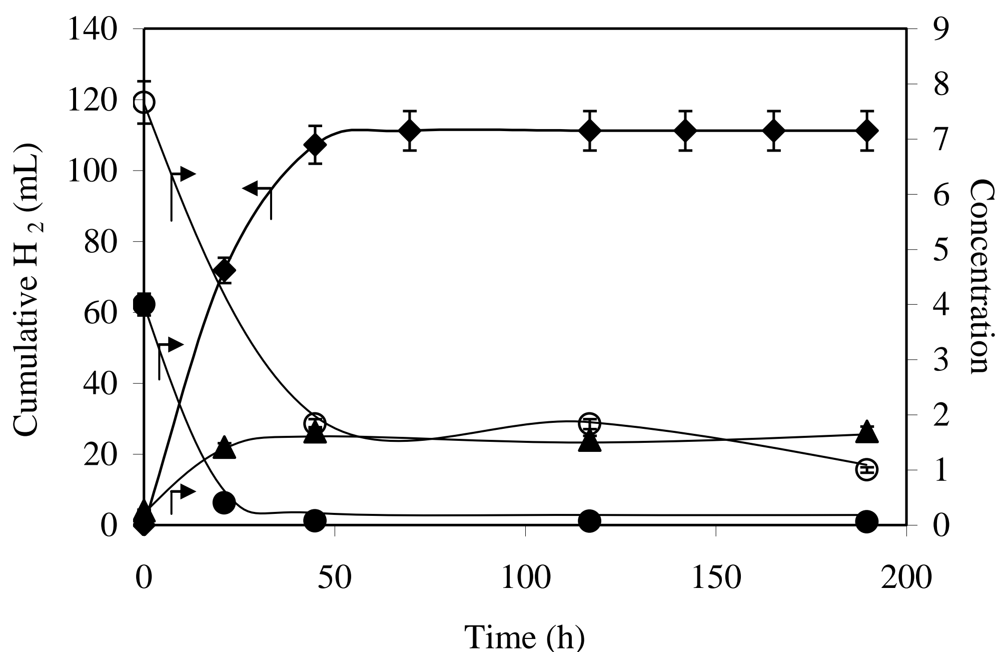


Figure 5.38 Variation of (♦) Cumulative hydrogen (mL), (▲) total volatile fatty acids (gL^{-1}), (●)glucose (gL^{-1}) and (○) NH_4-N (mg L^{-1}) concentrations with time for combined fermentation with halogen light source and 10 klux light intensity.

5.1.3.3.3 Effects of Lighting Period. Six batch combined fermentation experiments were performed with different durations of dark/light cycles using halogen lamp at 10 klux intensity. Figure 5.39 depicts variation of CHF with time for different durations of dark/light cycles. CHF increased with increasing durations of dark/light cycles from 0.5/0.5 to 6/6 h/h and reached the maximum level with continuous lighting (211 mL). Unlike the general trend, a decrease was observed in the CHF for 12/12 h/h dark/light cycle as compared to that of 6/6 h/h cycle.

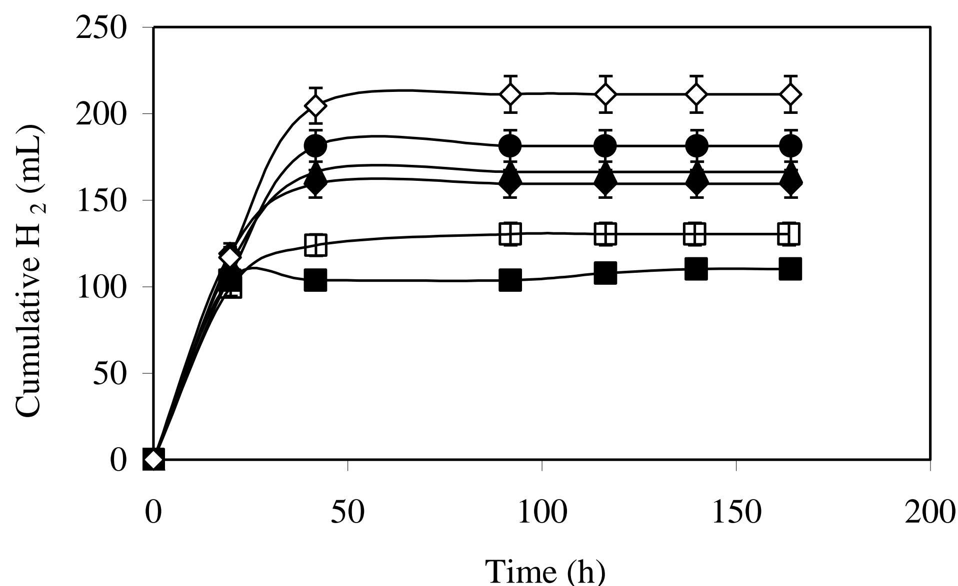


Figure 5.39 Variation of cumulative hydrogen formation with time for different durations of dark/light cycles in combined fermentation using halogen lamp with 10 klux intensity (■) 0.5/0.5, (♦) 2/2, , (▲) 4/4, (●) 6/6, (□) 12/12, (◊) Continuous.

The data presented in Figure 5.39 was correlated with the Gompertz equation, the constants were determined by regression analysis and are listed in Table 5.43 for different durations of dark/light cycles. Potential hydrogen formation (P) increased with increasing durations of dark/light cycle and became maximum with continuous lighting (211 mL). Maximum rate of hydrogen formation (R_m) did not present a general trend and varied between 5 and 6 mL h^{-1}. Durations of lag phases varied between 3 and 16 h with no regular trend.

Table 5.43 Gompertz equation constants for different durations of dark/light cycles in the combined fermentations with halogen lamp and 10 klux light intensity ($Starch_o$ = 5951 mgL^{-1})

Dark/Light Cycle (h/h)	**P (mL)**	**R_m (mL h^{-1})**	**λ (h)**	**R^2**
0.5/0.5	107.07	5.25	3.64	0.99
2/2	159.42	6.03	15.58	1.00
4/4	166.30	5.70	15.98	1.00
6/6	181.29	5.23	16.85	1.00
12/12	129.46	5.02	3.79	0.99
Continuous light	211.12	5.83	8.20	1.00

Hydrogen yields and specific production rates (SHPR) are presented in Table 5.44 for different durations of dark/light cycles. Hydrogen yields increased from 0.645 to 1.22 mol H_2 mol^{-1} glucose when dark/light cycle durations increased from 0.5/0.5 h/h to continuous lighting. A sudden drop was observed in the yield (0.766 mol H_2 mol^{-1} glucose) at 12/12 h/h cycle which may be due to inactivation of light fermentation bacteria for 12h unlighted period. SHPRs varied between 45 and 50 mL H_2 g^{-1} biomass h^{-1} with no general trend and the highest SHPR was obtained with continuous lighting (50 mL H_2 g^{-1} biomass h^{-1}). Initial starch, glucose and TVFA concentrations in all bottles were 5.95, 3.73, and 0.232 gL^{-1}, respectively. Final glucose and starch concentrations at the end of 163 h fermentation period were 0.24 and 0.07 gL^{-1}, respectively indicating nearly complete fermentation of starch and glucose to VFA, CO_2 and H_2 by the dark and light fermentation bacteria. However, final TVFA concentrations were nearly 3.1 ± 0.1 gL^{-1} indicating nearly 50% conversion of glucose to TVFAs. Final TVFA in continuous lighting was 2.58 gL^{-1} indicating hydrogen production by the light fermentation of TVFAs.

Dark fermentation took about 50 h for completion and both anaerobic sludge bacteria and *Rhodobacter* sp fermented glucose to hydrogen and TVFA. In dark fermentation period, *Rhodobacter* sp were adapted to glucose fermentation and became incapable of simultaneous fermentation of VFAs to hydrogen gas. In other words, presence of glucose caused ‘catabolite repression’ for the synthesis of enzymes required for VFA fermentation. Duration of the combined fermentation was not long enough (163 h) to allow *Rhodobacter* sp to switch their metabolism to fermentation of VFAs. In combined fermentation, longer fermentation periods (> 200 h) are required for fermentation of VFAs by the photo-fermentation bacteria after a long adaptation period (>100 h). Continuous lighting has enabled *Rhodobacter* sp to ferment part of the VFAs produced by dark fermentation yielding higher CHF as compared to the other lighting regimes. The ORP of the medium was around -175 mV at the beginning and decreased to nearly – 200 mV ± 50 mV at the end. Due to glucose consumption and VFA formation, medium pH decreased from 7.0 to about 5.8 at the first day of fermentation which was controlled manually around 6.5-7.0 for the fermentation period.

Table 5.44 Hydrogen yields and SHPR for different durations of dark/light cycles in combined fermentation with halogen lamp and 10 klux intensity ($Starch_o$ = 5951 mgL^{-1})

Dark/Light Cycle (h/h)	Yield ($mL\ H_2\ g^{-1}$ starch)	Yield ($molH_2mol^{-1}$ glucose)	SHPR ($mLH_2\ g^{-1}$ biomass h^{-1})
0.5/0.5	96.75	0.64	45.26
2/2	137.66	0.91	51.98
4/4	144.12	0.96	49.14
6/6	155.88	1.04	45.09
12/12	114.47	0.76	43.28
Continuous	182	1.21	50.26

There are no literature reports investigating the effects of light source, light intensity and lighting regime on performance of combined fermentation to compare our results with. Effects of light on hydrogen production were investigated only in light fermentations (Obeid et al., 2008; Shi et al., 2005; Tao et al., 2007; Uyar et al., 2007). The results of combined (this study) and sequential fermentation studies are compared in Table 5.45 in terms of CHF, hydrogen yield and SHPRs for the halogen lamp at the most suitable light intensity. As clearly shown in Table 5.45 sequential dark and light fermentations yielded higher CHFs and hydrogen yields as compared to combined fermentation. SHPR for combined fermentation was higher than that of sequential fermentation due to simultaneous hydrogen production from hydrolyzed starch by both dark and light fermentation bacteria. High hydrogen yields obtained with sequential dark-light fermentations is probably due to pre-treatment of dark fermentation effluent before light fermentation (ammonium removal, VFA adjustment by dilution, nutrient addition). In combined fermentation, adverse effects of light on dark fermentation bacteria, inhibitions caused by high concentrations of VFAs and NH_4-N on *Rhodobacter* sp and also different nutritional requirements and environmental conditions required by dark and light fermentation bacteria may have caused lower hydrogen yields. Therefore, sequential dark and light fermentations are recommended for high hydrogen yields and formation rates.

Table 5.45 Comparison of hydrogen production performances of combined and sequential dark and light fermentations at different light intensities

Fermentation scheme	**Light source**	**Light intensity**	**CHF (mL)**	**Hydrogen yield (mol H_2 mol^{-1} glucose)**	**SHPR (mL H_2 g^{-1} biomass h^{-1})**
Combined dark-light	Halogen lamp	270 Wm^{-2}	178	1.45	13
Sequential dark-light	Halogen lamp	5 klux	252	4.55	8.23

5.2 Experiments with Continuous Operation

5.2.1 Continuous Experiments for Combined Fermentation

5.2.1.1 Effects of Hydraulic Residence Time

Continuous combined fermentation experiments were performed at six (6) different hydraulic residence times (HRT) between 1 and 6 days which were established by changing the feed flow rate while keeping the fermentation volume at 2.554 litre constant level. Figure 5.40 depicts the variation of daily hydrogen gas production with different hydraulic residence times. The last three daily hydrogen volumes at steady-state for each HRT are presented. The daily hydrogen gas production decreased by increasing HRT. Highest hydrogen (365 mL) was produced when HRT was 1 day. This was followed with 275 $mLH_2\ d^{-1}$, 209 mLH_2d^{-1} and 182 mLH_2d^{-1} hydrogen production rates at HRT of 2, 3 and 4 days, respectively. The lowest daily hydrogen production was realized at HRT=6 days (170 mLH_2d^{-1}) which was almost the same that obtained at HRT = 5 days.

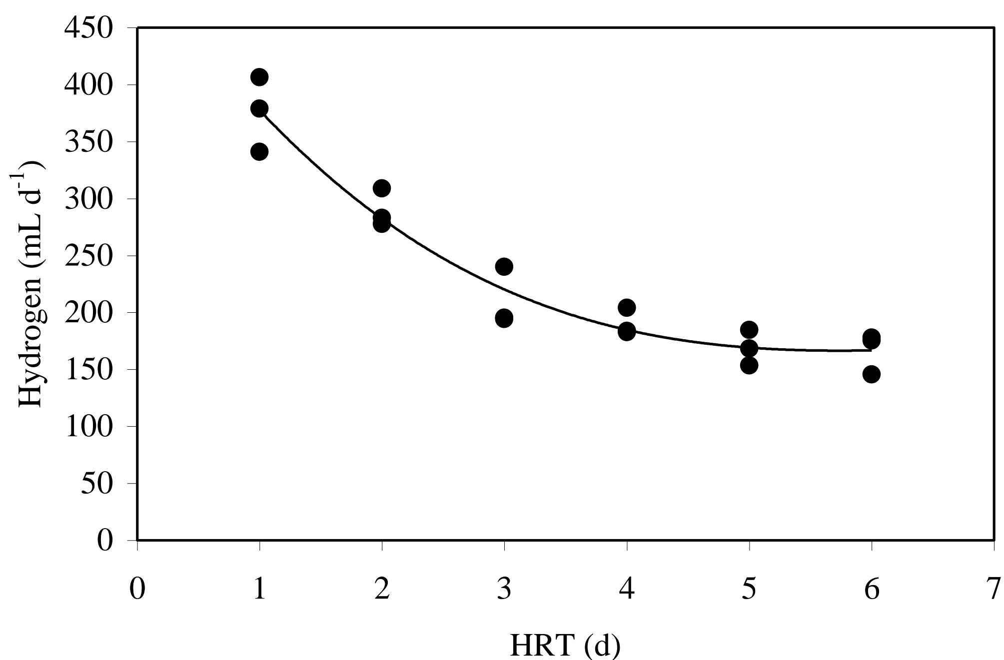


Figure 5.40 Variation of daily hydrogen gas production with hydraulic residence time

Variation of hydrogen production yield (mLH_2 g^{-1} starch) at different HRT levels is presented in Figure 5.41. Yields at steady-state for the last three days of operation were presented for each HRT. Hydrogen production yield increased with increasing HRT. The lowest and highest yields were 31 mLH_2 g^{-1} starch and 82 mLH_2 g^{-1} starch for HRT=1 day and HRT= 6 day, respectively. Trends in hydrogen production yield in Figure 5.41 and daily hydrogen production in Figure 5.40 are inversely related. High WP loadings at short HRT levels resulted in high volumes of hydrogen production, but low yields. The reason for low yields at short HRT levels might be insufficient time for fermentations.

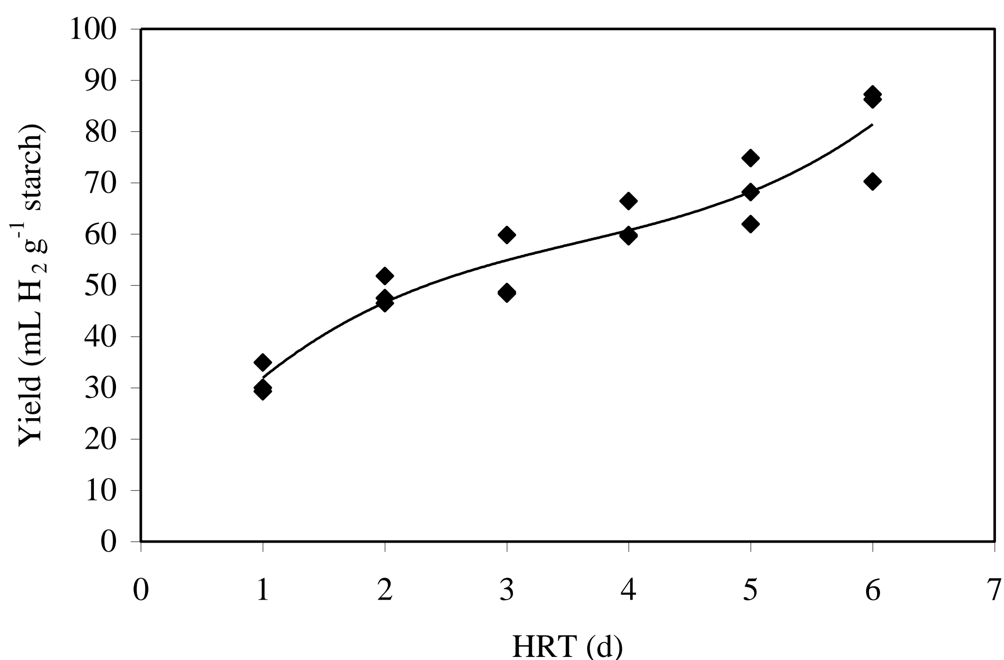


Figure 5.41 Variation of hydrogen production yield with hydraulic residence time

Figure 5.43 depicts variation of starch loading rate and percent starch utilization with hydraulic residence time. High percent starch consumptions were obtained at low starch loadings and vice-versa. Starch loading rate decreased with increasing HRT. The highest loading rate (12.77 g starch d^{-1}) was realized for HRT of 1day and lowest (2.13 g starch d^{-1}) was with 6 days of HRT. Starch loading rate decreased from 12.77 g starch d^{-1} to 6.38 g starch d^{-1}, 4.25 g starch d^{-1} , 3.19 g starch and to 2.55 g starch d^{-1} for 2 , 3 , 4 and 5 days of HRT operations, respectively. Starch utilization increased from about %90 to %97 when the HRT was increased from 1day to 6 days due to higher retention times in the reactor facilitating more efficient starch degradation and fermentation.

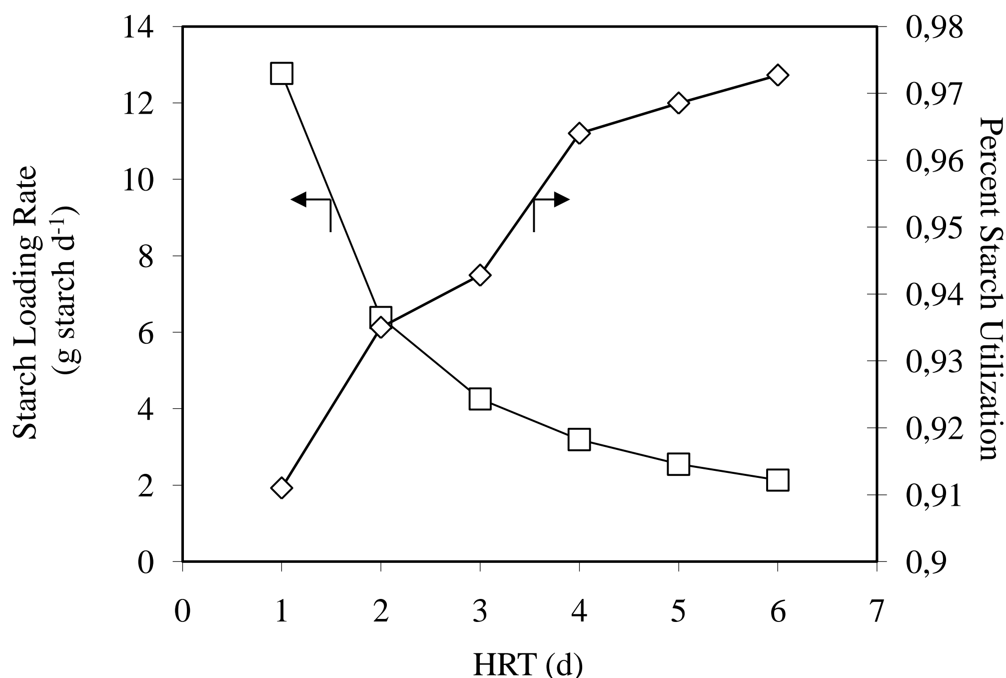


Figure 5.42 Variation of starch loading rate and percent starch utilization with hydraulic residence time

Variations of SHPR and VHPR with hydraulic residence time are presented in Figure 5.43. Both SHPR and VHPR decreased with increasing HRT. The highest SHPR of 9.16 mLH_2 g^{-1} cells h^{-1} was obtained with HRT=1 day operation. This was followed with 3.45 mLH_2 g^{-1} cells h^{-1} for HRT=2day. For the other HRT operations, SHPR remained almost constant at 3.5 mLH_2 g^{-1} cells h^{-1}. SHPR varies with biomass concentration in the reactor. Low SPHRs were obtained at high biomass concentrations since the SHPRs were calculated by dividing daily hydrogen gas production to the total amount of biomass (cell) inside the reactor at steady-state. The highest VHPR value (5.95 mLH_2 L^{-1} h^{-1}) was obtained with HRT=1 d since the highest amount of hydrogen was produced at that hydraulic residence time. This was followed by 4.5 mLH_2 L^{-1} h^{-1} VHPR at HRT=2 d operation. At higher HRT's the VHPR did not change much and remained around 2.8 mLH_2 L^{-1} h^{-1}.

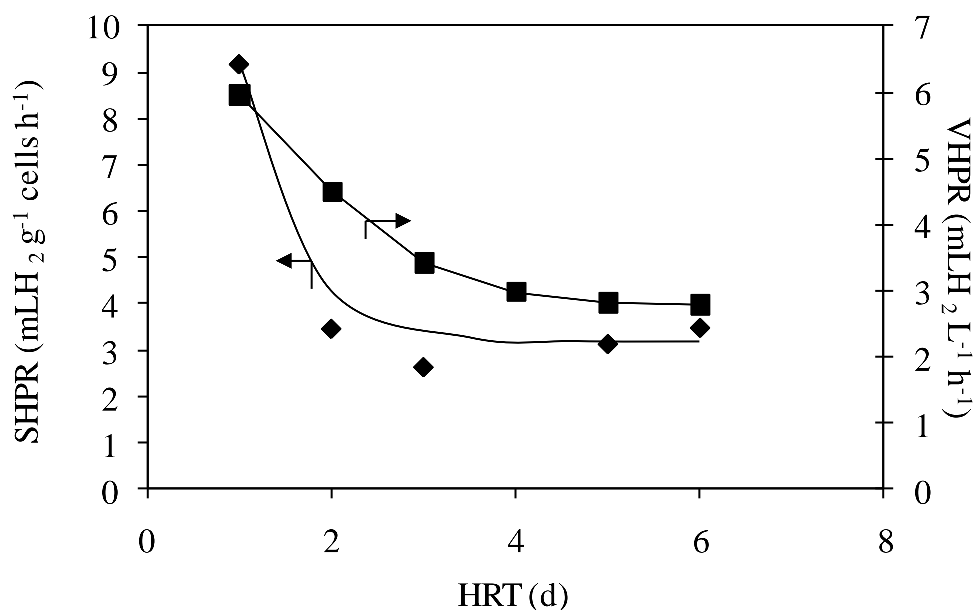


Figure 5.43 Variation of SHPR and VHPR with hydraulic residence time

Variations of daily hydrogen production (mLd^{-1}), starch (gL^{-1}), total volatile fatty acids (gL^{-1}), glucose (gL^{-1}) and NH_4-N (mgL^{-1}) concentrations with time for 6 day hydraulic residence time are depicted in Figure 5.44. Starch was effectively hydrolyzed by dark fermentation bacteria within the first day and decreased from 5 gL^{-1} to about 0.15 gL^{-1} and remained almost constant at steady-state. TVFAs produced by dark fermentation increased to 2.4 gL^{-1} within 2 days and remained almost constant for the rest of the fermentation period. Usually %60 of the starch is converted to TVFA during an effective dark fermentation. Therefore, it can be said that some amount of the TVFA was utilized by the light fermentative bacteria since the TVFA at steady-state was less then 3 gL^{-1}. Glucose concentration decreased from 1.1 gL^{-1} to about 50 mg L^{-1} within 24 h indicating effective fermentation of glucose by the dark and light fermentation bacteria for production of VFA, CO_2 and H_2. NH_4-N concentration was below 1 mgL^{-1} during the whole fermentation period and did not exceed the inhibition level for light fermentative bacteria. Daily hydrogen production increased from 0 to 200 mL within 24 h, and reached an avarege of 175 mLd^{-1} at steady-state conditions. pH and ORP of the medium varied between 7 and 6.4 and -130 mV ± 40, respectively. Decreases in pH occurred due to VFA formation and accumulation in the media.

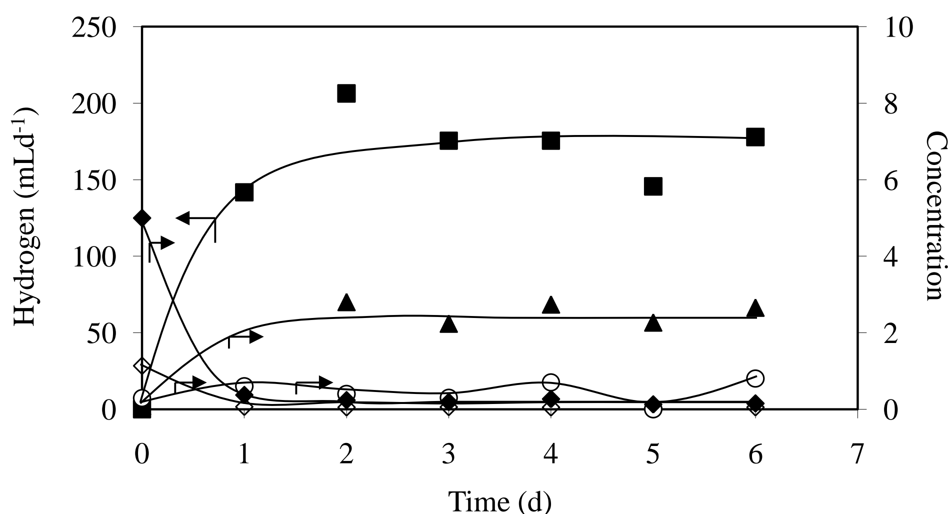


Figure 5.44 Variation of (■) daily hydrogen production (mL), (♦) starch (gL^{-1}), (▲) total volatile fatty acids (gL^{-1}), (◊) glucose (gL^{-1}) and (○) NH_4-N (mg L^{-1}) concentrations with time for combined fermentation in hybrid annular reactor for HRT=6 d.

Concentrations of starch (mgL^{-1}), TVFA (mgL^{-1}), glucose (mgL^{-1}), NH_4-N (mgL^{-1}) and biomass (gL^{-1}) at steady-state conditions for different HRT values between 1 and 6 days are presented in Table 5.46. The feed starch was nearly 4 gL^{-1}. Long retention times of the WPS in the reactor reduced the concentration of starch and increased hydrogen formation. Final starch concentration decreased with increasing HRT from 1 to 6 days due to longer hydrolysis time at high HRTs. The highest starch concentration (455 mgL^{-1}) was observed at HRT of 1 d. Final starch concentration decreased gradually with increasing HRT and the lowest starch concentration was obtained at HRT = 1 day (136.5 mgL^{-1}). Final TVFA concentrations also decreased with increasing HRT due to fermentation of VFAs by the photo-fermentative bacteria. TVFA concentration decreased from 2.85 gL^{-1} to 2.47 gL^{-1} when HRT increased from 1 d to 6 days. Glucose was effectively utilized during fermentation at all HRT levels and its concentration varied between 20-50 mgL^{-1}. NH_4-N concentration was lower than 1 mgL^{-1} for all HRT levels and was below the inhibitory level. Biomass concentration at steady state for HRT=1 day was 0.65 gL^{-1} which increased to 1.3 gL^{-1} at HRT =2 and HRT =3 days. Cell washout at low HRTs

reduced steady-state cell concentrations. Cell concentrations decreased to 0.9 gL^{-1} and 0.8 gL^{-1} at HRT= 5days and HRT=6 days, respectively.

Table 5.46 Starch, TVFA, glucose, NH_4-N and biomass concentrations for different hydraulic residence time operations at steady-state condition for continuous combined fermentation in hybrid annular bioreactor. Starcho = 5 gL^{-1}

HRT	$Starch_F$ (mgL^{-1})	$TVFA_F$ (mgL^{-1})	$Glucose_F$ (mgL^{-1})	NH_4-N_F (mgL^{-1})	$Biomass_F$ (gL^{-1})
1	455.00	2850.00	31.52	0.20	0.65
2	325.00	2817.00	28.80	0.30	1.30
3	286.00	2707.00	23.00	0.33	1.30
4	180.00	2775.00	25.00	0.10	-
5	157.42	2546.50	27.85	0.90	0.90
6	136.50	2471.50	48.90	0.70	0.80

Variation of VFA profile with HRT at steady-state conditions are presented in Table 5.47. Individual VFA and TVFA concentrations were measured with HPLC and analytical kits, respectively as explained in section 3.1.3. The summation of individual VFAs are not representing the TVFA since unknown peaks were obtained during HPLC analysis. This was the reason of measuring TVFA concentrations separately.

Table 5.47 VFA profile for different HRT levels.

HRT (d)	HAc (mgL^{-1})	HPr (mgL^{-1})	HBu (mgL^{-1})	HLa (mgL^{-1})
1	773.55	1160.20	-	-
2	994.25	1329.05	-	-
3	663.60	1108.45	-	958.00
4	788.45	1159.40	-	794.15
5	1190.25	1129.15	-	-
6	1039.40	981.00	-	-

Propionic acid (HPr) was the dominating VFA during all HRT levels with concentrations of about 1100 mgL^{-1}. Acetic acid (HAc) concentration increased from 773 mgL^{-1} to about 1100 mgL^{-1} by increasing the HRT from 1 to 6 days. Hydrogen formation yield showed an increasing trend with the increasing HRT due to high acetic acid concentrations at high HRTs. Butyric acid (HBu) was not detected in either one of different HRT operations. Lactic acid (HLa) concentrations were 958 mgL^{-1} and 794 mgL^{-1} at 3 and 4 days of HRT.

Formation and fermentation of VFAs along with hydrogen formation is a result of dark and light fermentations in combined fermentation. In theory VFAs produced in dark fermentation should simultaneously be converted to H_2 and CO_2 by light fermentative bacteria and the overall yield is expected to be as 1822.1 mL H_2 g^{-1} starch (at 30^oC and 1 atm) when only acetic acid is the intermediary product. However, formation of a mixture of different VFAs (HAc, HBu, HPr, HLa), and especially H_2 consuming propionic acid (HPr) production adversely effected H_2 production yield. At low HRTs the dominant anaerobic bacteria was propionic acid producers which consumed hydrogen to produce HPr and therefore, reduced the yield. At high HRTs (5-6 days) acetic acid producing bacteria became dominant producing hydrogen gas which resulted in high yields. Apparently anaerobic bacteria were acetate/propionate producers and the relative VFA concentrations varied with HRT along with changes in bacterial composition. Another reason for dominant HPr production might be a shift in dark fermentation metabolism from butyrate/acetate to acetate/propionate since the pH was kept between 7-7.5 (Hussy et al., 2007). The reason for no butyric acid formation might be due to lack of metabolic capabilities of *Clostridium beijerinkii* for Hbu formation. Butyric acid might have been deposited in form of PHB. Lactic acid was probably not produced by the *Clostridium* sp used or readily fermented by the photo-fermentation bacteria. At low HRTs (1-3days), VFAs produced by *Clostridium sp* were not fermented by *Rhodobacter sp*. resulting in low hydrogen yields due to insufficient fermentation times. However, at high HRTs (5-6 days) VFAs produced by dark fermentation were fermented to H_2 and CO_2 by *Rhodobacter sp* resulting in high yields. Accumulation of TVFA at short HRTs and low hydrogen yields indicate ineffectiveness of light fermentation at low HRTs. Major hydrogen production took place by fermentation of starch/glucose by *Clostridium and Rhodobacter sp* during the initial stages of fermentation. Hydrogen production through VFA fermentation by *Rhodobacter* sp took place to a limited extent at high HRTs.

No studies were reported in literature on continuous combined fermentation to date. Therefore, our results could not be compared with other literature studies.

CHAPTER SIX

CONCLUSIONS

Bio-hydrogen production from wheat powder starch (WPS) by batch and continuously operated dark and light fermentations were investigated in this study. Continuous experiments were performed after determination of optimum operating conditions in batch experiments. Batch and continuous combined dark-light fermentation experiments were also realized as an alternative to sequential dark and light fermentations. Selection of the most effective microbial cultures and optimization of environmental conditions for efficient hydrogen gas production from WPS were the major objectives of this thesis.

6.1 Batch Experiments

6.1.1 Batch Dark Fermentation Experiments

Bio-hydrogen production performances of different pure and mixed anaerobic cultures, *Clostridium butyricum, Clostridium acetobutylicum, Enterobacter aerogenes,* heat-treated anaerobic sludge and a mixture of the cultures were investigated in batch dark fermentation of wheat powder solution (WPS). Boiled wheat powder solution containing 10 gL^{-1} WP was used in batch experiments without any nutrient (N, P) and trace element addition. Heat-treated anaerobic sludge (ANS) resulted in the highest CHF (560 mL H_2), hydrogen yield (223 mL H_2 g^{-1} starch = 1.14 mol H_2 mol^{-1}glucose) and SHPR (32.1mL H_2 g^{-1} h^{-1}) among the other cultures tested. Cumulative hydrogen formation by the *E. aerogenes* (545 mL H_2) was comparable with the ANS. However, the rate (10.4 mL H_2 g^{-1} biomass h^{-1}) and the yield of hydrogen formation (159 mL H_2 g^{-1} starch = 1.04 mol H_2 mol^{-1} glucose) by *E. aerogenes* were lower than that of the ANS culture. Heat treated anaerobic sludge was found to be the most effective bio-hydrogen prıoducer among the other cultures tested.

Hydrogen consuming methanogens were eliminated and spore forming hydrogen producers were selected by different pre-treatment methods. Hydrogen formation

performances of heat and chloroform pre-treated anaerobic sludges from different sources were compared by batch dark fermentation experiments using 20gL^{-1} wheat powder as the substrate. Among the pre-treatment methods tested, 10 h (2x 5h) boiled PAK sludge resulted in the highest CHF (652 mL H_2), hydrogen yield (150.85 mL H_2 g^{-1} starch =1mol H_2 mol^{-1} glucose) and SHPR (25.71 mL H_2 g^{-1} cells h^{-1}). When a granular sludge was subjected to 10 h boiling, lower hydrogen yields were obtained due to heat penetration problems inside the granules. Reducing the heat treatment time from 10h to 5h did not affect hydrogen production significantly. Chloroform treatment alone did not eliminate hydrogen consumers. Heat pre-treatment was more effective than chloroform treatment. Combinations of heat and chloroform treatments were also not as effective as the repeated-heat treatment.

Partial hydrolysis of wheat starch by boiling before fermentation improved the rate of hydrogen production and reduced the lag phase duration. Boiled and unboiled WPS resulted in 446 mL and 433 mL cumulative hydrogen gas at the end of experiments. However, hydrogen production was faster with the boiled WPS. This is due to formation of readily fermentable glucose from partial hydrolysis of starch. The SHPR for boiled and unboiled WPS were 55.17 mL H_2 g^{-1} biomass h^{-1} and 46.62 mL H_2 g^{-1} biomass h^{-1} respectively.

Hydrogen production by dark fermentation of WPS using heat treated anaerobic acidogenic sludge was investigated at different medium compositions by changing initial C/N and C/P ratios in order to determine the N and P requirements of the organisms for effective hydrogen production. A Box-Wilson statistical experiment design was used by considering C/N (20-200) and C/P (50-1000) ratio as independent variables while hydrogen formation yield and the rate were the objective functions. The experimental data was correlated with a quadratic response function and the coefficients were determined by regression analysis. The response function predictions were in good agreement with the experimental data for hydrogen yield. The hydrogen yield increased with increasing C/N and C/P ratio yielding the maximum yield (23 mg H_2 g^{-1}starch or 281 mL H_2 g^{-1} starch at STP) at C/N = 200 and C/P = 1000. The SHPR also increased with increasing C/N and C/P ratios yielding the highest SHPR (8.0 mg H_2 g^{-1}biomass h^{-1} or 98 mL H_2 g^{-1}biomass h^{-1} at

STP) at C/N = 200 and C/P = 1000. The C/N/P ratio yielding the highest hydrogen yield (23 mg H_2 g^{-1} starch) and formation rate (8.0 mg H_2 g^{-1}biomass h^{-1}) was C/N/P = 100/0.5/0.1. The results indicated low nitrogen and phosphorous requirements (N/ P/ starch = 0.2/0.04/100, w w^{-1} w^{-1}) for high hydrogen yields and formation rates by dark fermentation of WPS.

Effects of the initial substrate (WP starch) and biocatalyst (biomass) concentrations on the rate and the yield of hydrogen and TVFA formation were investigated in batch fermentation of powdered wheat by heat treated anaerobic sludge. Cumulative hydrogen formation increased with the initial WP concentration and reached a maximum level at 20 gL^{-1} WP concentration. The hydrogen yield was maximum and the TVFA yield was minimum at WP concentration of 20 gL^{-1} indicating a shift in the metabolic pathway or in the composition of the bacterial flora depending on the WP concentration. SHPR also increased with the initial WP concentration and became maximum at 20 gL^{-1} WP concentration. High WP concentrations such as 30 gL^{-1} resulted in a decrease in the SHPR due to substrate inhibition.

Biomass concentration also affected the yield and the rate of hydrogen and TVFA formation. Cumulative hydrogen formation increased with increasing initial cell concentration up to 2.5 gL^{-1} and reached the highest level. The hydrogen yield was maximum and the TVFA yield was minimum at the cell concentration of 2.5 gL^{-1}. The optimum biomass/ substrate ratio maximizing the hydrogen yield and formation rate was determined to be 0.125 g biomass g^{-1} WP. A kinetic model was developed for bio-hydrogen formation from wheat starch and the constants were determined by regression analysis. Wheat-starch concentrations above 30 gL^{-1} was found to reduce the hydrogen formation rate considerably.

6.1.2 Batch Light Fermentation Experiments

Hydrogen gas production by light fermentation of dark fermentation effluent of wheat powder (WP) solution was realized by using different *Rhodobacter sphareoides (RS)* cultures and their mixtures. The pure RS-NRRL culture resulted in the highest cumulative hydrogen (48 mL H_2) and the formation rate (1.52 mL H_2 h^{-1})

while the highest yield (250 mL H_2 g^{-1} TVFA) was obtained with the pure RS-RV culture. Mixtures of the *Rhodobacter* cultures improved the hydrogen yield and the formation rate considerably since different cultures used different VFAs in the mixture. A mixture of the three *Rhodobacter* cultures resulted in the highest yield of 693 mL H_2 g^{-1} TVFA in the light fermentation.

A mixture of three *Rhodobacter* cultures was used to determine the optimum TVFA and NH_4-N concentrations yielding the highest rate and extent of hydrogen formation. Low concentrations of TVFA (<2350 mgL^{-1}) and NH_4-N (<47 mgL^{-1}) resulted in substrate limitations, while high concentrations (TVFA$>$ 2350 mgL^{-1} and NH_4-N $>$ 47 mgL^{-1}) caused substrate inhibition yielding the optimum concentrations as 2350 mg TVFA L^{-1} and 50 mg NH_4-N L^{-1}, respectively.

The effects of initial biomass concentration on light fermentative hydrogen gas production was investigated by varying the initial cell concentration between 0.5-5 gL^{-1}. The cumulative hydrogen production was low for high initial cell concentrations above 1 gL^{-1}. Probably this was a result of the floc formation by bacteria which consequently caused a prevention of light penetration through the fermentation media. High hydrogen productions were realized with low initial biomass concentrations. High initial biomass concentrations resulted in high TVFA consumption up to % 73, but low hydrogen production which might be a result of the utilization of the TVFA for other cellular functions and deposition in form of PHB. The highest yield and SHPR were obtained with 0.5 gL^{-1} initial biomass concentration as 387 mL H_2 g^{-1}TVFA and 10.66 mL H_2 g^{-1} biomass h^{-1}.

Bio-hydrogen production by the light fermentation of dark fermentation effluent of wheat powder solution (WPS) was investigated using different light sources and light intensities. Both the light source and the intensity affected the rate and the yield of hydrogen gas production by *R.sphaeroides*-RV. The most suitable light source was found to be halogen lamp yielding 781 mL H_2 g^{-1} TVFA with a SHPR of 17.5 mL H_2 g^{-1} biomass h^{-1}. The optimum light intensity was determined to be 5 klux (approx 176 W m^{-2}) yielding 1037 mL H_2 g^{-1} TVFA a with a SHPR of 8.23 mL H_2 g^{-1} biomass h^{-1} when halogen lamp was used as the source of light. The total yield of hydrogen by the dark and light fermentations was 4.55 moles H_2 mol^{-1} glucose when

halogen lamp was used with 5 klux light intensity. Hydrogen production was limited by the availability of the light up to 5 klux and was inhibited by high light intensities above 5 klux.

6.1.3 Batch Combined Fermentation Experiments

Hydrogen production by combined dark-light fermentation of ground wheat solution was realized at different dark/light biomass ratios (D/L) between 1/2 and 1/10 along with only dark and light fermentations. Light and dark fermentations alone resulted in the higher cumulative hydrogen production (98 mL) within 200 h. However, in combined fermentations the highest rate (12.17 mL H_2 g^{-1} biomass h^{-1}) and the yield (176 mL H_2 g^{-1} starch) of hydrogen formation were obtained with a D/L ratio of 1/7. At low D/L ratios, hydrogen formation rate was limited by the low concentration of the dark fermentation bacteria. At high D/L ratios such as D/L = 1/10 hydrogen formation from VFAs produced by dark fermentation was limited by the low concentration of light fermentation bacteria. The optimal D/L ratio resulting in balanced VFA formation and fermentation rates by the dark and light fermentations was 1/7. Combined dark-light fermentation of ground wheat for hydrogen production was found to be advantageous resulting in faster hydrogen formation rates as compared to dark and light fermentations alone.

Ground wheat solution was subjected to dark-light combined fermentation in the same reactor for bio-hydrogen production at different wheat powder (substrate) and cell concentrations. In variable substrate concentration experiments, the highest cumulative hydrogen (135 mL) and formation rate (3.44 mL H_2 h^{-1}) were obtained with the 20 gL^{-1} wheat powder concentration while the 2.5 gL^{-1} wheat concentration resulted in the highest yield (64 mL H_2 g^{-1} starch). When the cell concentration was changed between 0.5 and 5 gL^{-1} the wheat powder concentration was constant (5 gL^{-1}) with a D/L ratio of 1/7. The highest cumulative hydrogen (118 mL) and yield (157 mL H_2 g^{-1} starch) were obtained with the 1.1 gL^{-1} cell concentration yielding an optimal cell/ substrate ratio of 0.22 g cells/g WP. It is more advantageous to operate the combined fermentation at low wheat powder concentrations (<10 gL^{-1}) with the optimum cell concentration to obtain high cumulative hydrogen formation (118 mL)

and high hydrogen yields (156 mL H_2 g^{-1} starch). High wheat powder concentrations cause substrate and product (VFA) inhibitions reducing the rate and the yield of bio-hydrogen formation. High cell concentrations cause bacterial floc formation and inavalability of the insoluble starch inside the flocs resulting in low rates and yields for starch hydrolysis and hydrogen formation.

Effects of light source, intensity and lighting period on bio-hydrogen production by combined dark and light fermentation of ground wheat starch was investigated. A mixture of heat treated anaerobic sludge and *R. sphaeroides*-RV was used in batch experiments. Both the light source and the intensity affected the rate and the yield of hydrogen gas production in combined fermentation. The most suitable light source was found to be halogen lamp yielding 178 mL CHF and 218 mLH_2 g^{-1} starch hydrogen yield with a rather slow hydrogen formation (1.34 mL h^{-1}). The rate and the yield of hydrogen formation increased with increasing Light intensity and reached the highest level (4.73 mL h^{-1} and 139.7 mLH_2 g^{-1} starch) at 10 klux with halogen lamp. Hydrogen production was limited by the availability of light inside the rather dark combined fermentation medium. Effects of different dark/light cycle durations were also invstigated and continuous lighting was found to be the most suitable lighting regime with the highest CHF (211 mL) and hydrogen yield (1.22 mol H_2 mol^{-1} glucose). Hydrogen yields and rates obtained in combined fermentation were lower than those obtained with the sequential dark and light fermentations.

6.2 Continuous Experiments

6.2.1 Continuous Combined Fermentation Experiments

Continuous combined fermentation experiments in a hybrid annular bio-reactor were performed at six (6) different hydraulic residence times (HRT) between 1 and 6 days by changing the feed flow rate while keeping the fermentation volume at 2.554 litre constant level. Pure cultures of *Clostridium beijerinkii* DSMZ 791 and *Rhodobacter sphaeroides*-RV were used. High hydrogen production rates (mL d^{-1}) were observed at low HRT operations due to high WP loadings into the reactor. In contrast hydrogen production yield increased with increasing HRT due to longer

fermentation times at high HRTs. The highest daily hydrogen production (365 mL H_2 d^{-1}) and highest yield (82 mLH_2 g^{-1} starch) were obtained for HRT=1 day and HRT=6 days, respectively. The extent of starch utilization increased with decreasing WP loading rate and increasing HRT. Up to 97% starch utilization was obtained at HRT=6 days. Both SHPR and VHPR decreased with increasing HRT. The highest SHPR of 9.16 mLH_2 g^{-1} cells h^{-1} was obtained with HRT of 1 day. Long HRT operations yielded more effective starch and TVFA fermentation and prevented TVFA accumulation in the media. Also low biomass concentrations such as 0.8 gL^{-1} at HRT=6 days is more favorable in terms of more effective light penetration through the medium. Operation of continuous combined fermentation at high HRTs (5-6 days) is more advantageous due to fermentation of VFAs by photo-fermentation for improved bio-hydrogen production.

Highest yields and specific hydrogen production rates regarding relevant fermentation schemes that were obtained during this study are summarized in Table 6.1. As clearly shown in Table 6.1 sequential dark and light fermentation resulted in highest hydrogen formation yield followed by dark and combined fermentations respectively. Dark fermentation yielded highest SHPR among other fermentations because of the presence of readily available glucose in its fermentation media and presence of any non-inhibiting conditions. SHPR for combined fermentation was higher than that of sequential fermentation due to simultaneous hydrogen production from hydrolyzed starch by both dark and light fermentation bacteria. High hydrogen yields obtained with sequential dark-light fermentations is probably due to pre-treatment of dark fermentation effluent before light fermentation (ammonium removal, VFA adjustment by dilution, nutrient addition). In combined fermentation, adverse effects of light on dark fermentation bacteria, inhibitions caused by high concentrations of VFAs and NH_4-N on *Rhodobacter* sp and also different nutritional requirements and environmental conditions required by dark and light fermentation bacteria may have caused lower hydrogen yields. Low yields were also obtained during combined continuous experiments probably due to same reasons in batch combined fermentation experiments. Dark fermentation resulted in quite high SHPR and yield but the effluent still contained remarkable TVFA. Therefore, sequential

dark and light fermentations are recommended for high hydrogen yields and formation rates.

Table 6.1 Comparison of hydrogen production performances of different batch fermentation schemes.

Batch Fermentation scheme	**Type of culture**	**Yield ($molH_2$ mol^{-1} glucose)**	**SHPR (mLH_2 g^{-1} biomass h^{-1})**
Dark	ANS	1.87	295.45
Sequential Dark-Light	RS-RV	4.55	8.23
Combined Dark -Light	ANS + RS-RV	1.45	13

Recommendations for future studies

Some of the recommendations for future studies are listed below:

- Different kinds of operation modes such as sequencing batch can be used for dark and light fermentations of WPS for hydrogen production.
- Biofilm or fluidized bed reactors may be used for dark and light fermentations
- New isolated or genetically modified microorganisms capable of producing hydrogen gas more effectively can be utilized.
- Hydrogen impermeable reactors equipped with online pH, ORP, temperature, light control units may be used
- Optimization of medium composition especially for photo-fermentation to improve hydrogen formation should be realized.
- Dark fermentative cultures producing only acetic and butyric acids should be developed.
- More effective pre-treatment methods to remove hydrogen consuming homoacetogens from anaerobic sludge should be developed.
- In order to prevent inhibitory concentrations of VFA in dark and combined fermentation, simultaneous removal of VFAs and hydrogen gas should be achieved.
- More effective light fermentation bacteria should be developed in order to increase the rate of hydrogen formation.
- Hydrogen production capabilities of natural microorganisms present in wheat powder should be investigated.

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APPENDICES

RAW EXPERIMENTAL DATA AND FIGURES

A.1 Raw Data for Batch Experiments

A.1.1 Raw Data for Dark Fermentative Hydrogen Production

A.1.1.1 Raw Data for Microbial Culture Selection

Table A. 1 Raw data for Anaerobic Sludge (ANS)

Date	**Clock**	**Time(h)**	**V_l(mL)**	**V_g (mL)**	**$C_{H2.i}$ (%)**	**$C_{H2.i-1}$ (%)**	**Vw (mL)**	**V_{H2} (mL)**	**pH**	**ORP(mV)**	**Glucose (gL^{-1})**	**Starch (gL^{-1})**	**TVFA (gL^{-1})**
27.06.2007	17:00	0	400	730	0.00	0.00	0	0	7.00	-200	0.88	6.80	0.81
27.06.2007	20:26	3.43	354	776	0.00	0.00	0.10	0					
28.06.2007	10:13	17.22	354	776	0.38	0.38	0	2.96	4.84	-20	1.07		
28.06.2007	16:50	23.83	327	803	1.70	1.70	0.10	13.66					
29.06.2007	08:56	39.93	315	815	5.25	5.00	0.10	42.80	4.76	-108	4.89		
29.06.2007	16:40	47.56	300	830	5.10	5.00	0	44.38					
30.06.2007	08:37	63.6	300	830	7.38	7.28	0	64.13	4.91	-36	4.51		
01.07.2007	08:39	87.63	270	860	8.90	8.90	0	80.25	5.00	-340			
02.07.2007	08:45	111.73	253	877	14.40	13.20	0.05	130.00	5.34	-200	2.49	3.50	
03.07.2007	08:24	135.44	230	900	25.70	6.80	0	245.54					
03.07.2007	10:37	137.64	230	900	26.00	25.00	180	465.14	5.48	-500	0.35		
04.07.2007	08:57	159.97	209	921	25.00	25.00	0	470.39	6.34	-445	0.12		
05.07.2007	08:32	183.55	194	936	25.00	25.00	0	474.14	6.36	-347	0.34		
06.07.2007	08:32	207.55	176	954	25.00	17.60	0	478.64	6.40	-350	0.39		
07.07.2007	08:30	231.52	165	965	25.00	24.00	0	551.99	6.40	-402	0.40		
09.07.2007	08:54	279.92	150	980	24.60	23.60	0	561.47	6.40	-220	0.38		
10.07.2007	08:41	303.7	135	995	23.60	21.80	0	565.01	6.40	-326	0.28		
11.07.2007	10:50	329.8	120	1010	21.80	21.00	0	568.28	6.50	-300	0.33	0.42	2.81

Table A. 2 Raw data for Mixed Culture (MIX)

Date	Clock	Time(h)	V_l (mL)	V_g (mL)	$C_{H2.i}$ (%)	$C_{H2.i-1}$ (%)	Vw (mL)	V_{H2} (mL)	pH	ORP(mV)	Glucose (gL^{-1})	Starch (gL^{-1})	TVFA (gL^{-1})
27.06.2007	17:00	0	400	730	0.00	0.00	0	0	7.00	-45	0.70	8.82	0.62
27.06.2007	20:30	3.49	379	751	0.00	0.00	0	0					
28.06.2007	10:25	17.42	375	755	0.00	0.00	0	0	4.37	0	0.60		
28.06.2007	17:11	24.18	350	780	0.32	0.32	0	2.53					
29.06.2007	09:05	40.07	350	780	0.36	0.36	0	2.82	4.75	-60	0.51		
29.06.2007	16:45	47.74	321	809	0.67	0.65	0	5.40					
30.06.2007	08:45	63.73	319	811	1.80	1.80	0	14.74	5.66	-56	2.80		
01.07.2007	08:49	87.8	289	841	6.78	6.50	0.10	57.17	5.00	-340			
02.07.2007	08:57	111.9	268	862	17.30	14.50	0	151.63	4.98	-440	2.21	3.30	
03.07.2007	08:37	135.6	241	889	31.00	30.00	153.50	349.81	5.45	-500	0.21		
04.07.2007	09:12	160.2	235	895	30.60	30.40	0	356.98	6.32	-425	0.05		
05.07.2007	08:36	183.4	218	912	30.40	29.50	0	362.15	6.40	-355			
06.07.2007	08:37	207.4	206	924	29.50	21.40	0	365.69	6.41	-305	0.37		
07.07.2007	08:35	231.4	190	940	28.00	27.40	0	431.15	6.47	-418	0.35		
09.07.2007	08:59	279.8	174	956	27.40	25.80	0	435.54	6.45	-133	0.37		
10.07.2007	08:46	303.6	156	974	26.00	25.00	0	442.13	6.50	-202	0.25		
11.07.2007	08:55	329.8	141	989	25.00	23.90	0	445.88	6.60	-215	0.27	0.42	4.11

Table A. 3 Raw data for CAB

Date	Clock	Time(h)	V_l (mL)	V_g (mL)	$C_{H2.i}$ (%)	$C_{H2.i-1}$ (%)	Vw (mL)	V_{H2} (mL)	pH	ORP(mV)	Glucose (gL^{-1})	Starch (gL^{-1})	TVFA (gL^{-1})
27.06.2007	17:00	0	400	730	0.00	0.00	0	0	7.00	-100	0.68	9.20	0.38
27.06.2007	20:20	3.33	388	742	0.00	0.00	1.60	0					
28.06.2007	08:57	15.95	388	742	0.00	0.00	0	0	4.50	-3	1.00		
28.06.2007	16:40	23.66	357	773	0.38	0.37	0	2.93					
29.06.2007	09:25	40.41	357	773	0.53	0.53	0	4.19	4.30	-120	2.13		
29.06.2007	16:37	47.61	320	810	1.14	1.09	0.10	9.31					
30.06.2007	08:56	63.92	307	823	5.60	5.50	0	46.57	5.20	-75	4.52		
01.07.2007	08:34	87.55	295	835	10.50	10.10	0	88.98	5.00	-210			
02.07.2007	08:37	111.6	266	864	20.30	19.70	0	180.04	5.10	-425	2.21	3.05	
03.07.2007	08:17	135.27	237	893	31.00	29.00	284	374.70	5.54	-448	0.16		
03.07.2007	16:49	143.82	237	893	31.00	30.00	0	392.56					
04.07.2007	08:42	159.7	229	901	30.00	29.00	4.20	396.22	6.43	-432			
05.07.2007	08:24	183.4	215	915	31.00	30.00	0	418.58	6.37	-417			
06.07.2007	08:26	207.4	196	934	30.00	29.00	0	424.28	6.43	-267	0.48		
07.07.2007	08:21	231.32	186	944	29.00	28.00	0	427.18	6.45	-400	0.37		
09.07.2007	08:46	279.74	167	963	28.00	26.00	0	432.50	6.47	-188	0.36		
10.07.2007	08:35	303.55	152	978	26.00	24.80	0	436.40	6.47	-222	0.27		
11.07.2007	10:39	329.62	135	995	24.80	24.20	0	440.61	6.47	-200	0.30	0.44	4.40

Table A. 4 Raw data for CB

Date	Clock	Time(h)	V_l (mL)	V_g (mL)	$C_{H2.i}$ (%)	$C_{H2.i-1}$ (%)	Vw (mL)	V_{H2} (mL)	pH	ORP(mV)	Glucose (gL^{-1})	Starch (gL^{-1})	TVFA (gL^{-1})
27.06.2007	17:00	0	400	730	0.00	0.00	0	0	7.0	-40	0.82	7.80	0.43
27.06.2007	20:32	3.53	368	762	0.00	0.00	0	0					
28.06.2007	10:27	17.45	368	762	0.00	0.00	0	0	4.30	-176	0.66		
28.06.2007	17:03	24.04	340	790	0.00	0.00	0	0					
29.06.2007	08:58	39.96	340	790	0.00	0.00	0	0	4.70	-32	0.42		
29.06.2007	16:48	47.79	316	814	0.00	0.00	0	0		-47			
30.06.2007	09:04	64.05	316	814	0.46	0.44	0	3.71	5.80	-438	0.38		
01.07.2007	08:57	87.93	283	847	2.45	2.43	0.10	20.85	5.80	-252			
02.07.2007	08:50	111.82	258	872	6.80	5.60	0.20	59.57	5.52	-252	4.21	6.13	
03.07.2007	08:30	135.84	235	895	5.60	4.90	0	60.86	5.96	-500	2.19		
03.07.2007	10:47	137.76	235	895	9.00	8.80	0	97.56					
04.07.2007	09:05	160.1	210	920	11.00	10.00	7.30	120.80	6.10	-500	2.42		
05.07.2007	08:44	183.75	196	934	14.00	13.00	2	159.84	5.91	-420	1.38		
06.07.2007	08:46	207.76	134	996	13.00	9.20	0	167.90	6.26	-507			
07.07.2007	08:42	231.69	121	1009	17.60	17.60	19.10	257.21	6.46	-500	0.36		
09.07.2007	09:09	280.14	117	1013	17.60	13.00	7.60	259.02	6.49	-205	0.05		
10.07.2007	08:50	303.8	102	1028	19.60	19.60	0	328.82	6.48	-245	0.05		
11.07.2007	11:03	329.9	98	1032	19.60	18.80	0	329.60	6.50	-150	0.06	0.91	2.66

Table A. 5 Raw data for EA

Date	Clock	Time(h)	V_l (mL)	V_g (mL)	$C_{H2.i}$ (%)	$C_{H2.i-1}$ (%)	Vw (mL)	V_{H2} (mL)	pH	ORP(mV)	Glucose (gL^{-1})	Starch (gL^{-1})	TVFA (gL^{-1})
27.06.2007	17:00	0	400	730	0.00	0.00	0	0	7.00	-60	0.67	9.33	0.29
27.06.2007	20:34	3.56	376	754	0.00	0.00	0	0					
28.06.2007	10:10	17.17	376	754	0.00	0.00	0	0	4.80	-40	0.83		
28.06.2007	17:10	24.16	335	795	0.00	0.00	0	0					
29.06.2007	09:15	40.24	335	795	2.00	2.00	0.10	15.90	4.67	-266	3.00		
29.06.2007	16:32	47.52	310	820	4.30	4.10	0.05	35.26					
30.06.2007	08:50	63.78	300	830	7.50	7.26	0.1	63.90	5.10	-63	4.58		
01.07.2007	09:05	88.07	283	847	12.30	11.50	0.1	107.83	5.10	-387			
02.07.2007	09:05	112.06	251	879	15.00	14.60	0.05	142.28	5.42	-400	3.40	5.13	
03.07.2007	09:10	136.06	232	898	19.80	19.60	41.80	200.03	5.65	-420	1.37		
04.07.2007	09:32	160.5	211	919	22.50	21.90	37.20	239.17	6.34	-365	1.96		
05.07.2007	08:49	183.78	202	928	23.00	20.00	2.70	251.97	6.30	-340	1.47		
06.07.2007	08:53	207.85	198	932	23.00	23.00	0	280.73	6.12	-450	1.71		
07.07.2007	08:47	231.75	181	949	25.00	12.40	2.50	304.24	5.84	-400	1.20		
09.07.2007	09:16	280.23	151	979	26.50	17.40	6.70	447.78	6.07	-215	0.52		
10.07.2007	08:58	303.93	143	987	25.70	25.70	1	531.35	6.00	-290	0.39		
11.07.2007	11:07	329.96	125	1005	25.70	25.40	0	535.97	6.00	-109	0.27	0.89	3.15

A.1.1.2 Raw Data for Selection of Sludge Pre-treatment Method

Table A. 6 Raw data for 10 h boiled PAK

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-50	16.97
20.08.2008	09:40	15.50	285	845	10.00	9.57	120	96.5	4.95	-56	
20.08.2008	14:56	20.76	279	851	24.60	24.20	219	278.85	5.77		
21.08.2008	09:49	39.59	273	857	38.60	36.50	465	583.20	5.80	-32	
21.08.2008	15:16	45.03	266	864	38.90	37.10	10	610.38	6.70		
22.08.2008	11:26	65.39	265	865	37.10	37.10	100	644.28	6.45		
25.08.2008	09:42	135.46	263	867	37.10	37.10	43	657.74	5.90	-110	
26.08.2008	09:21	159.11	253	877	37.10	37.10	0	657.74	6.10	-158	
27.08.2008	14:58	176.43	251	879	37.10	37.10	0	657.74	6.25	-185	
28.08.2008	15:39	201.39	248	882	37.10	37.10	0	657.74	6.15	-232	1.67

Table A. 7 Raw data for 10 h boiled EFES

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-47	17.10
20.08.2008	09:44	15.56	285	845	2.48	2.48	70	22.69	5.60	-110	
20.08.2008	15:01	20.83	281	849	8.39	8.32	60	78.00	5.35		
21.08.2008	09:54	39.73	277	853	12.80	11.20	12.30	118.17	4.60	-27	
21.08.2008	15:18	45.13	269	861	12.70	12.70	10	133.20	6.50		
22.08.2008	11:43	65.55	268	862	12.70	12.10	0	133.33	5.92		
25.08.2008	09:45	135.58	265	865	16.00	15.00	225	203.42	6.80	-112	
26.08.2008	09:24	159.23	255	875	15.00	15.00	55	210.79	6.99	-135	
27.08.2008	15:01	176.83	253	877	15.00	15.00	2.40	211.10	7.00	-165	
28.08.2008	15:41	201.20	250	880	15.00	15.00	0	211.10	6.80	-245	2.53

Table A. 8 Raw data for 10 h boiled MIX

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-41	17.93
20.08.2008	09:48	15.63	285	845	0.45	0.45	43	3.99	6.30	-109	
20.08.2008	15:03	20.88	283	847	3.13	3.13	30	27.64	5.60		
21.08.2008	09:57	39.73	280	850	8.42	8.36	80	79.43	4.50	-35	
21.08.2008	15:22	45.14	273	857	8.36	8.36	2.20	80.20	6.15		
22.08.2008	11:47	65.56	272	858	8.36	8.36	0	80.28	5.48		
25.08.2008	09:49	135.59	269	861	27.20	26.70	365	342.08	6.80	-117	
26.08.2008	09:27	159.23	259	871	26.70	26.70	30	349.02	6.91	-147	
27.08.2008	15:03	176.83	257	873	26.70	26.70	0	349.02	6.87	-179	
28.08.2008	15:44	201.51	254	876	26.70	26.70	0	349.02	6.70	-262	2.53

Table A. 9 Raw data for only %0.05 Chloroform treated MIX

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-52	18.34
20.08.2008	09:51	15.68	285	845	0.00	0.00	60	0	4.40	-80	
20.08.2008	15:06	20.93	280	850	0.32	0.31	0	2.69	4.73		
21.08.2008	10:00	39.83	276	854	0.32	0.31	0	2.75	5.00	-85	
21.08.2008	15:25	45.25	269	861	0.32	0.32	0	2.82	6.79		
22.08.2008	11:51	65.93	268	862	0.35	0.33	0	3.11	6.30		
25.08.2008	09:53	135.97	266	864	11.40	10.80	310	134.08	6.80	-172	
26.08.2008	09:30	159.58	256	874	10.80	10.80	20	135.47	7.00	-190	
27.08.2008	15:07	177.18	254	876	10.80	10.80	0	135.47	6.96	-232	
28.08.2008	15:46	201.83	251	879	10.80	10.80	0	135.47	6.80	-290	2.29

Table A. 10 Raw data for 0.05% chloroform treatment after 10h boiling of MIX

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-57	17.83
20.08.2008	09:54	15.76	285	845	0.00	0.00	40	0	4.80	-77	
20.08.2008	15:09	20.98	281	849	0.00	0.00	0	0	4.96		
21.08.2008	10:02	39.98	278	852	0.00	0.00	0	0	5.00	-75	
21.08.2008	15:27	45.43	269	861	0.00	0.00	0	0	6.92		
22.08.2008	11:55	65.90	268	862	0.32	0.32	0	2.75	6.25		
25.08.2008	09:56	135.91	266	864	25.00	25.00	420	321	7.00	-153	
26.08.2008	09:33	159.53	256	874	25.00	25.00	70	336.75	7.24	-160	
27.08.2008	15:10	177.15	254	876	25.00	25.00	3.70	337.53	7.10	-194	
28.08.2008	15:49	201.80	251	879	25.00	25.00	0	337.53	6.95	-263	2.48

Table A. 11 Raw data for 5h boiling after 0.05% chloroform treatment of MIX

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-62	19.30
20.08.2008	09:57	15.81	285	845	11.50	11.50	208	121.09	4.50	-95	
20.08.2008	13:12	19.06	280	850	14.20	14.20	38	150.01	6.02		
21.08.2008	10:06	39.96	277	853	21.70	21.30	175	252.39	5.00	-130	
21.08.2008	15:30	45.36	271	859	21.30	21.00	0	253.67	6.90		
22.08.2008	11:59	65.85	269	861	21.50	21.50	0	258.39	6.41		
25.08.2008	10:01	135.86	267	863	22.80	22.50	240	324.76	6.47	-190	
26.08.2008	09:37	159.46	257	873	22.50	22.50	20	328.96	6.71	-195	
27.08.2008	15:13	177.06	255	875	22.50	22.50	3	329.54	6.70	-251	
28.08.2008	15:52	201.71	252	878	22.50	22.50	0	329.54	6.55	-282	2.16

Table A. 12 Raw data for 5h boiling after 0.1% chloroform treatment of MIX

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i (%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP	Starch (gL^{-1})
19.08.2008	18:10	0.00	285	845	0.00	0.00	0	0	7.00	-50	19.07
20.08.2008	10:00	15.83	285	845	2.36	2.36	60	21.35	6.15	-112	
20.08.2008	15:15	21.08	283	847	5.97	5.97	55	55.26	5.83		
21.08.2008	10:09	39.98	280	850	13.10	13.10	140	134.38	4.50	-33.5	
21.08.2008	15:33	45.38	275	855	13.10	13.10	0	135.04	6.50		
22.08.2008	12:04	65.90	274	856	13.10	13.10	0	135.17	5.22		
25.08.2008	10:05	135.90	271	859	22.10	19.30	262	270.78	6.10	-152	
26.08.2008	09:40	159.48	261	869	25.40	25.00	230	329.20	6.75	-145	
27.08.2008	15:17	177.10	259	871	25.00	25.00	40	338.60	6.84	-185	
28.08.2008	15:54	201.70	256	874	25.00	25.00	0	338.60	6.68	-209	2.51

A.1.1.3 Raw Data for Investigating the of Effects of Boiling of WPS

Table A. 13 Raw data for boiled WPS

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i/%)	CH_2,i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (gL^{-1})	TVFA (gL^{-1})
24.09.2009	15:00	0.00	290	840	0.00	0.00	0	0	7.00	-300	1512	7.91	0.39
25.09.2009	09:26	18.43	290	840	23.80	23.80	723.40	372.08	5.20	-178	583	2.28	
26.09.2009	11:04	25.63	272	858	27.00	27.00	107	432.71	6.15	-220	154	2.12	
28.09.2009	09:34	72.13	260	870	22.90	22.90	60	446.45					
29.09.2009	09:24	95.96	260	870	16.00	16.00	0	446.45	6.37	-300	107	0.57	4.81

Table A. 14 Raw data for unboiled WPS

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2,i/%)	CH_2,i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (gL^{-1})	TVFA (gL^{-1})
24.09.2009	15:00	0.00	290	840	0.00	0.00	0	0	7.00	-300	396	8.13	0.37
25.09.2009	09:30	18.50	290	840	14.80	14.80	345.80	175.49	6.30	-258	198	5.71	
26.09.2009	11:07	25.61	276	854	29.20	29.20	455	433.40	5.65	-181.1	98	1.46	
28.09.2009	09:35	72.07	266	864	20.80	20.80	0	433.40					
29.09.2009	09:26	95.92	266	864	14.60	14.60	0	433.40	6.80	-405	61	0.73	4.34

A.1.1.4 Raw Data for Investigating the of Effects of C/N and C/P Ratios

Table A. 15 Raw data for A1

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
01.08.2007	11:54	0	970	1280	0.39	0.39	0	0	7.0	-40	3332.70	16888	1740
02.08.2007	08:55	22	950	1300	11.60	11.60	272.40	177.36	4.6	-193			
02.08.2007	16:12	29.12	900	1350	11.80	7.94	40	184.78	5.7				
03.08.2007	08:47	45.7	900	1350	16.50	13.30	120	320.14	4.2	-17	7652.60		
03.08.2007	15:51	52.76	875	1375	15.00	14.70	0	343.52	6.1				
04.08.2007	09:21	70.26	875	1375	14.70	13.20	0	343.52	5.1	-128			
06.08.2007	08:39	117.56	850	1400	21.40	11.80	260	513.96	5	-130	3572.24		
07.08.2007	08:45	141.66	815	1435	19.30	18.70	0	621.58	5.7	-177		5464	5620
08.08.2007	08:59	165.89	800	1450	21.90	21.90	17	671.70	6.3	-203	1155.40		
09.08.2007	08:42	189.6	785	1465	21.90	8.27	1047	901.00	6.3	-250			
10.08.2007	08:37	213.5	750	1500	30.00	27.00	527	1385.05	6.3	-179	308.37		
11.08.2007	10:54	239.5	730	1520	28.90	27.50	180	1465.95	6.4	-198			
13.08.2007	08:30	285.1	710	1540	30.00	29.30	51	1519.75	6.5	-219	334.07		
14.08.2007	08:45	308.85	695	1555	29.30	29.30	0	1519.75	6.6	-124			
15.08.2007	08:28	332.56	670	1580	29.60	27.40	0	1524.49	6.7	-183	371.20	1560	8216

Table A. 16 Raw data for A2

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
01.08.2007	11:40	0	970	1280	0.37	0.37	0	0	7.00	-55.50	6069.70	22091	2136
02.08.2007	10:01	22.33	920	1330	10.50	10.50	210	156.79	5.28	-155.70			
02.08.2007	15:56	28.26	895	1355	14.70	14.30	40	219.58	5.84				
03.08.2007	09:33	46.28	895	1355	35.70	26.40	3580	1787.61	5.37	-345	3175.30	6582	
03.08.2007	15:40	52.4	860	1390	42.30	39.00	1585	2679.08	6.00				
04.08.2007	08:47	69.51	860	1390	40.80	17.00	1280	3226.34	5.96	-180			
06.08.2007	08:34	117.29	850	1400	33.70	27.30	0	3460.14	6.60	-202	415.50	1891	
07.08.2007	08:56	141.66	825	1425	30.00	30.00	0	3498.61	6.63	-192		1205	4796
08.08.2007	08:53	165.61	810	1440	30.00	8.34	0	3498.61	6.01	-120	376.57		
09.08.2007	08:51	189.61	790	1460	22.90	22.80	0	3711.19	5.48	-95		1713	
10.08.2007	08:44	213.51	760	1490	22.80	22.80	0	3711.19	6.17	-165.30	408.82		
11.08.2007	10:49	239.42	750	1500	22.80	22.80	0	3711.19	5.88	-142.50		1945	
13.08.2007	08:37	285.63	725	1525	22.80	22.80	0	3711.19	6.27	-165	383.40	1946	
14.08.2007	08:38	309.63	700	1550	22.80	22.80	0	3711.19	6.28	-151			
15.08.2007	08:43	333.56	680	1570	22.80	22.80	0	3711.19	6.24	-142.80	235.38	1610	9504

Table A. 17 Raw data for A3

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
01.08.2007	11:51	0	970	1280	2.00	2.00	0	0	7.0	-68	10159.57	18660	1880
02.08.2008	09:39	21.80	910	1340	14.50	14.50	302.50	211.36	5.0	-250			
02.08.2008	16:06	28.15	895	1355	21.50	19.40	190	347.06	5.7				
03.08.2008	09:11	45.23	895	1355	35.80	32.90	1611.50	1146.20	4.5	-124.40	4483.50		
03.08.2008	16:07	52.23	860	1390	32.90	30.40	0	1146.20	6.3				
04.08.2008	09:14	69.46	860	1390	32.40	28.00	40	1186.96	5.1	-205			
06.08.2007	08:56	117.16	850	1400	34.00	29.60	940	1590.56	6.3	-250	588.26		
07.08.2007	08:38	140.86	825	1425	34.20	25.00	360	1779.23	6.3	-144		2696	6592
08.08.2007	08:44	164.96	800	1450	25.00	17.00	60	1794.23	6.4	-140	305.12		
09.08.2007	09:03	189.27	775	1475	17.00	12.00	40	1801.03	6.4	-161.80			
10.08.2007	08:49	213.03	760	1490	26.20	14.30	0	2012.61	6.3	-170	286.68		
11.08.2007	10:45	238.96	750	1500	22.80	21.60	0	2140.11	6.1	-145.60			
13.08.2007	08:40	285.04	725	1525	21.60	21.60	0	2140.11	5.8	-140	287.90		
14.08.2007	08:42	309.04	700	1550	21.60	21.60	0	2140.11	6.3	-124.20			
15.08.2007	08:35	332.92	690	1560	21.60	21.60	0	2140.11	6.1	-147.30	272.17	770	10968

Table A. 18 Raw data for A4

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
20.09.2007	12:10	0	985	1265	0.00	0.00	0	0	7.00	-114	5746	16754	1516
21.09.2007	07:27	19.283	985	1265	0.45	0.38	8.50	5.70	3.89	-105			
21.09.2007	12:29	24.31	950	1300	0.55	0.51	5	7.91	6.26				
22.09.2007	15:05	50.91	950	1300	17.00	15.50	800	358.33	3.85	-110	9954		
24.09.2007	10:04	93.91	930	1320	20.00	18.70	115	440.73	4.20		4939	10534	
25.09.2007	09:06	116.91	890	1360	30.00	28.00	100	624.41	6.23	-162			
26.09.2007	09:11	140.89	860	1390	35.90	34.00	730	996.29	4.85	-150	885		
27.09.2007	09:08	164.84	860	1390	34.00	32.30	0	996.29	6.40	-185			
28.09.2007	09:10	188.87	850	1400	32.30	35.00	0.30	996.39	6.18	-355	784		
01.10.2007	09:09	260.87	830	1420	35.00	35.00	9	999.54	6.25	-360	376	2827	8712
02.10.2007	09:56	285.62	820	1430	35.00	35.00	537.50	1187.67	6.22	-283			
03.10.2007	08:51	308.53	810	1440	35.00	35.00	6.4	1189.91	6.10	-152	304		
04.10.2007	08:52	332.53	800	1450	35.00	35.00	1	1190.26	6.13	-121	318	2165	908

Table A. 19 Raw data for F1

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
01.08.2007	11:40	0	970	1280	0.32	0.32	0	0	7.00	-71	2368.60	17887	2460
02.08.2007	09:30	21.7	875	1375	7.92	7.90	132	114.91	6.00	-170.80			
02.08.2007	15:42	27.9	850	1400	11.00	10.50	20	160.51	6.50				
03.08.2007	09:24	45.6	850	1400	21.10	20.40	645	445.00	4.51	-100	8561.90		
03.08.2007	16:02	52.2	805	1445	21.90	19.30	0	466.68	6.00				
04.08.2007	08:59	69.2	805	1445	34.80	23.00	2608.50	1598.46	5.50	-310			
06.08.2007	08:44	92.95	760	1490	46.80	40.60	3080	3394.47	6.13	-220	948.05		
07.08.2007	08:49	141.03	750	1500	40.60	36.30	165	3461.46	5.91	-145		1358	5876
08.08.2007	08:49	165.03	710	1540	36.30	9.89	0	3461.46	6.47	-136	481.18		
09.08.2007	08:37	188.81	700	1550	19.00	19.00	0	3602.67	6.38	-187.70			
10.08.2007	08:52	213.07	675	1575	30.00	28.00	0	3775.92	6.26	-148	365.07		
11.08.2007	10:59	238.97	650	1600	28.00	27.70	0	3775.92	6.12	-151			
13.08.2007	08:49	285.13	630	1620	27.70	27.70	0	3775.92	5.62	-115	415.17		
14.08.2007	08:49	309.13	610	1640	27.70	27.70	0	3775.92	6.26	-123.70			
15.08.2007	08:39	332.96	590	1660	27.70	27.70	0	3775.92	5.95	-141.50	335.75	1260	10755

Table A. 20 Raw data for F2

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
17.09.2007	12:00	0	980	1270	0.00	0.00	0	0	7.0	-96	7474	14800	3230
18.09.2007	07:32	19.53	980	1270	0.00	0.00	22.80	0	4.0	2			
18.09.2007	14:44	26.73	970	1280	0.31	0.31	10.60	3.98	4.8				
19.09.2007	07:18	43.3	960	1290	2.40	1.50	198	35.69	5.1	-485	8301		
19.09.2007	13:57	49.95	945	1305	22.30	22.30	520	423.09	4.8				
20.09.2007	07:17	67.28	945	1305	29.80	27.40	550	684.86	4.4	-201			
20.09.2007	13:54	73.9	925	1325	27.40	14.30	0	684.86	6.0				
21.09.2007	07:21	91.35	925	1325	14.30	10.79	7.70	685.96	4.8	-182	3325		
21.09.2007	12:32	96.53	910	1340	15.80	12.70	0	753.10	6.5				
22.09.2007	15:18	123.3	910	1340	27.60	23.30	210	1010.72	5.8	-310			
24.09.2007	09:56	165.9	875	1375	32.70	21.50	160	1192.29	6.8	-200	611.7	3198	7676
25.09.2007	09:27	189.5	850	1400	27.30	25.60	350	1369.04	6.5	-217			
26.09.2007	09:22	213.4	835	1415	25.60	22.10	71	1387.22	6.5	-155	229		
27.09.2007	09:24	237.4	825	1425	22.10	20.00	0	1387.22	6.3	-165			
28.09.2007	09:21	261.4	800	1450	20.00	20.00	0	1387.22	6.3	-125	246		
01.10.2007	09:26	333.4	790	1460	20.00	20.00	0	1387.22	6.2	-109	243.7	1767	10104

Table A. 21 Raw data for F3

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
01.08.2007	11:43	0	970	1280	0.37	0.37	0	0	7.00	-36.50	8137.90	18688	2216
02.08.2007	09:52	22.4	925	1325	8.40	8.40	170	120.62	5.42	-220			
02.08.2007	16:00	28.5	900	1350	13.50	13.50	28	193.25	5.90				
03.08.2007	08:52	45.36	900	1350	30.40	35.40	2221	1275.13	4.68	-183	5744.90		
03.08.2007	15:56	52.32	875	1375	25.70	31.90	8.50	1298.47	6.03				
04.08.2007	09:25	69.81	875	1375	23.80	29.30	40	1359.69	4.87	-130			
06.08.2007	09:05	117.47	850	1400	19.11	23.80	480	1473.93	6.30	-160	899.50		
07.08.2007	08:59	141.37	840	1410	19.11	19.11	60	1485.40	6.22	-158		2445	7048
08.08.2007	08:38	165.04	810	1440	19.11	19.11	20	1489.22	6.05	-134.40	580.97		
09.08.2007	08:56	189.3	800	1450	19.11	19.11	0	1489.22	5.79	-142.60			
10.08.2007	08:58	213.34	750	1500	19.11	19.11	0	1489.22	6.09	-138	540.68		
11.08.2007	10:41	239.05	725	1525	19.11	19.11	0	1489.22	5.73	-142.50			
13.08.2007	08:44	285.1	700	1550	19.11	19.11	0	1489.22	6.00	-146	428.50		
14.08.2007	08:53	309.25	690	1560	19.11	19.11	0	1489.22	5.96	-128.80			
15.08.2007	08:32	332.9	660	1590	19.11	19.11	0	1489.22	6.49	-175.60	361.40	1830	11725

Table A. 22 Raw data for F4

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
17.09.2007	12:00	0	980	1270	0.00	0.00	0	0	7.0	-59	9603	13200	1670
18.09.2007	07:20	19.33	980	1270	0.32	0.31	24	4.10	3.9	-22			
18.09.2007	14:41	26.68	970	1280	0.37	0.36	13	4.87	5.0				
19.09.2007	07:15	43.25	950	1300	2.50	2.20	370	41.92	5.0	-305	7429		
19.09.2007	13:47	49.47	945	1305	23.00	22.50	440.30	414.64	5.0				
20.09.2007	07:11	66.87	945	1305	30.00	29.60	520	668.51	4.3	-149			
20.09.2007	14:02	73.69	925	1325	29.60	26.60	0	668.51	6.3				
21.09.2007	07:31	93.206	925	1325	27.50	27.50	8.80	682.86	4.9	-156	2078		
21.09.2007	12:25	98.11	900	1350	27.50	14.20	0	682.86	6.7				
22.09.2007	15:26	125.12	900	1350	33.90	32.70	430	1094.60	6.5	-156			
24.09.2007	09:46	167.45	890	1360	32.70	32.70	810	1359.40	6.6	-256	366	2338	6436
25.09.2007	09:21	191.03	860	1390	32.70	31.90	480	1516.40	6.5	-319			
26.09.2007	09:08	214.81	850	1400	31.90	30.00	110	1551.50	6.6	-335	332		
27.09.2007	09:01	238.7	825	1425	30.00	29.60	25.50	1559.10	6.7	-334			
28.09.2007	09:14	262.92	810	1440	29.70	28.90	6	1562.40	6.7	-404	348		
01.10.2007	09:20	335.02	800	1450	28.90	27.90	0	1562.40	6.6	-439	342.87	1961	10092

Table A. 23 Raw data for centre point (Data are given in average)

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH_2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH_2 (mL)**	**pH**	**ORP (mV)**	**Glucose (mgL^{-1})**	**Starch (mgL^{-1})**	**TVFA (mgL^{-1})**
17.09.2007	12:00	0	980	1270	0.00	0.00	0	0	7.00	-69	8885	16100	3655
18.09.2007	07:02	19	980	1270	0.00	0.00	13.60	0	3.88	-13			
18.09.2007	15:06	27.1	960	1290	0.32	0.32	10	4.147	4.85				
19.09.2007	07:10	43.16	960	1290	12.10	2.60	233.30	184.38	5.03	-322	10239		
19.09.2007	14:07	50.09	950	1300	22.90	22.90	360	530.72	4.85				
20.09.2007	07:27	67.44	950	1300	29.00	27.80	470	746.32	4.26	-206			
20.09.2007	13:50	73.82	920	1330	27.80	27.80	0	746.32	6.06				
21.09.2007	07:04	91.057	920	1330	27.80	25.60	16.20	750.83	4.45	-136	2123		
21.09.2007	12:37	96.67	880	1370	25.60	19.30	0	750.83	6.58				
22.09.2007	15:22	123.42	870	1380	28.40	27.50	175	926.11	6.13	-375			
24.09.2007	10:00	166.05	860	1390	33.00	31.50	500	1167.56	6.20		596	2589	5664
25.09.2007	09:10	189.22	850	1400	31.50	29.80	130	1208.51	5.95	-236			
26.09.2007	09:20	213.38	840	1410	29.70	28.00	13	1210.96	6.66	-257	561		
27.09.2007	09:11	237.23	825	1425	29.60	28.90	13.10	1237.64	6.62	-216			
28.09.2007	09:23	261.43	810	1440	28.90	26.30	9.30	1240.33	6.48	-390	276		
01.10.2007	09:17	333.3	790	1460	26.30	26.30	0	1240.33	6.48	-360	254	1930	9744

Table A. 24 Raw data for no N and P addition

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
17.09.2007	12:00	0	980	1270	0.00	0.00	0	0	7.0	-43	8380	22300	1880
18.09.2007	07:07	19.1	980	1270	0.00	0.00	42.40	0	3.8	7			
18.09.2007	14:59	27	965	1285	0.32	0.32	8	4.13	4.7				
19.09.2007	07:24	43.4	965	1285	7.54	7.54	220	113.50	5.5	-519	8620		
19.09.2007	14:03	50.05	950	1300	14.30	14.10	100	215.68	6.1				
20.09.2007	07:22	67.04	950	1300	23.50	21.60	540	464.78	4.3	-129			
20.09.2007	13:57	73.62	925	1325	21.60	9.70	0	464.78	5.7				
21.09.2007	07:07	90.97	925	1325	12.10	8.80	34.50	500.76	5.1	-187	6700		
21.09.2007	12:21	96.003	900	1350	25.00	25.00	0	719.46	6.6				
22.09.2007	15:11	122.84	900	1350	32.30	30.00	755	1061.90	5.7	-335			
24.09.2007	09:39	165.31	890	1360	35.00	31.20	1110	1518.40	5.5		556	4092	5160
25.09.2007	09:14	188.89	870	1380	36.00	34.50	0	1584.60	6.5	-289			
26.09.2007	09:29	213.14	860	1390	34.50	33.60	1.90	1585.30	6.3	-245	411		
27.09.2007	09:24	237.05	850	1400	33.60	32.40	4.30	1586.70	6.7	-185			
28.09.2007	09:05	260.73	830	1420	32.40	31.30	7	1589.00	6.6	-245	323		
01.10.2007	09:15	332.9	805	1445	31.30	31.30	0	1589.00	6.5	-230	289	1955	8118

A.1.1.5 Raw Data for Initial Substrate and Biomass Concentrations

Table A. 25 Raw data for 5 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
04.02.2008	16:30	0.00	385	223	0.00	0.00	0	0	7.0	-205	363.50	5026	390
05.02.2008	09:07	16.60	385	223	4.52	4.00	22	11.07	5.4	-247			
06.02.2008	09:34	41.05	379	229	26.60	21.90	105	90.99	4.0	-161	410.10		
07.02.2008	09:12	64.68	358	250	31.00	31.00	80	143.15	5.5	-267			
08.02.2008	09:12	88.68	343	265	33.00	25.60	0	153.10	6.5	-325	31.95		
09.02.2008	13:49	117.29	332	276	34.80	34.80	0	181.31	6.7	-400			
11.02.2008	09:24	160.88	322	286	34.80	34.80	0	184.79	6.6	-379			
13.02.2008	09:14	208.71	321	287	34.80	34.80	0	185.13	6.7	-372	33.95		
14.02.2008	09:35	233.06	311	297	34.80	34.80	0	188.61	6.8	-385	41.94	322.5	2740

Table A. 26 Raw data for 10 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
04.02.2008	16:30	0.00	390	218	0.00	0.00	0	0	7.0	-196	1448.67	10300	590
05.02.2008	09:12	16.70	390	218	6.63	6.63	40	17.10	3.6	-155			
06.02.2008	09:29	40.98	376	232	16.40	14.50	10	42.34	4.8	-225	937.37		
07.02.2008	09:21	64.68	356	252	38.90	30.00	235	198.14	4.9	-275			
08.02.2008	09:27	88.95	341	267	51.70	39.20	150	338.13	5.9	-356	183.74		
09.02.2008	13:52	117.37	330	278	39.20	39.20	25	351.12	6.0	-375			
11.02.2008	09:21	160.85	320	288	39.20	39.20	0	355.04	6.3	-350			
13.02.2008	09:10	208.67	319	289	39.20	39.20	0	355.43	6.5	-386	135.81		
14.02.2008	09:30	233.00	309	299	44.10	44.10	0	374.00	6.6	-423	109.84	581.24	4570

Table A. 27 Raw data for 15 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
04.02.2008	16:30	0.00	390	218	0.00	0.00	0	0	7.0	-195	2673.65	14340	670
05.02.2008	09:23	16.88	390	218	7.45	7.45	61	20.78	4.3	-205			
06.02.2008	09:23	40.88	388	220	46.50	37.70	312	251.92	3.9	-186	3568.41		
07.02.2008	09:38	65.13	369	239	43.60	39.00	180	351.67	4.5	-262			
08.02.2008	09:21	88.85	354	254	46.00	37.00	145	442.00	6.1	-324	283.60		
09.02.2008	13:18	116.80	343	265	37.70	37.70	25	457.17	6.1	-393			
11.02.2008	09:04	160.50	333	275	37.70	37.70	0	460.94	6.0	-315			
13.02.2008	09:05	208.50	332	276	37.70	37.70	0	461.32	6.3	-328	147.13		
14.02.2008	09:38	233.05	322	286	42.40	42.40	0	478.53	6.5	-350	175.09	887.68	5500

Table A. 28 Raw data for 20 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	Starch (mgL^{-1})	TVFA (mgL^{-1})
04.02.2008	16:30	0.00	390	218	0.00	0.00	0	0	7.0	-205	3411.96	20911	480
05.02.2008	09:28	16.96	390	218	6.90	6.13	50	18.49	3.7	-240			
06.02.2008	09:40	41.16	383	225	45.30	32.00	320	252.01	4.4	-265	4473.83		
06.02.2008	16:16	47.75	362	246	61.90	61.90	120	406.57	4.8				
07.02.2008	09:02	64.48	362	246	61.90	61.90	430	600.07	4.6	-225			
08.02.2008	09:32	88.81	347	261	61.90	61.90	190	675.47	5.9	-300	619.14		
09.02.2008	13:37	116.90	336	272	72.80	72.80	65	737.21	6.0	-392			
11.02.2008	09:13	160.48	336	272	72.80	72.80	35	745.02	6.1	-355			
13.02.2008	09:17	208.50	335	273	79.30	79.30	0	763.49	6.4	-334	168.43		
14.02.2008	09:27	233.66	325	283	79.30	79.30	0	771.42	6.6	-380	177.08	1260.20	7330

Table A. 29 Raw data for 30 gL^{-1} initial WP concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH_2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH_2 (mL)**	**pH**	**ORP (mV)**	**Glucose (mgL^{-1})**	**Starch (mgL^{-1})**	**TVFA (mgL^{-1})**
04.02.2008	16:30	0.00	390	218	0.00	0.00	0	0	7.0	-217	4553.72	29500	650
05.02.2008	09:33	17.05	390	218	13.30	13.20	70	38.30	3.6	-188			
06.02.2008	09:10	40.66	379	229	23.00	19.70	60	75.99	4.0	-196	3115.70		
07.02.2008	09:17	64.77	357	251	39.00	33.70	240	222.38	4.9	-275			
08.02.2008	09:06	88.58	336	272	52.00	40.00	340	456.03	5.0	-265	1248.94		
09.02.2008	13:41	117.16	322	286	43.00	37.80	320	607.81	6.3	-410			
11.02.2008	09:16	160.74	312	296	37.80	37.80	70	631.89	6.2	-375			
13.02.2008	09:21	208.82	311	297	37.80	37.80	9.50	634.00	6.5	-374	499.31		
14.02.2008	09:43	233.68	301	307	44.50	44.50	0	658.35	6.7	-380	345.52	2214.40	11700

Table A. 30 Raw data for 0.5 gL^{-1} initial biomass concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH_2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH_2 (mL)**	**pH**	**ORP (mV)**	**Starch (mgL^{-1})**	**TVFA (mgL^{-1})**
15.04.2008	16:15	0.00	192	416	0.00	0.00	0	0	7.00	-126.50	17970	530
16.04.2008	09:12	16.95	192	416	0.72	0.72	10.80	3.07	3.50	-60		
16.04.2008	15:23	23.13	187	421	0.76	0.76	1.50	3.28	4.80			
17.04.2008	09:06	40.85	180.5	427.5	1.10	1.10	0	4.79	4.20	-110		
18.04.2008	09:15	65.00	174.1	433.9	1.79	1.79	0	7.85	4.40	-111.60		
19.04.2008	10:54	90.65	171.1	436.9	7.97	7.49	130	45.27	5.80	-228		
21.04.2008	09:18	137.05	167.5	440.5	7.49	7.49	0	45.54	6.80	-300	2280	6600

Table A. 31 Raw data for 1.5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Starch (mgL^{-1})	TVFA (mgL^{-1})
15.04.2008	16:15	0.00	192	416	0.00	0.00	0	0	7.00	-145	17430	530
16.04.2008	09:15	17.00	192	416	12.20	10.00	209.10	76.26	3.60	-122		
16.04.2008	15:28	23.21	186	422	15.00	14.80	45	104.71	4.50			
17.04.2008	09:09	40.90	181.5	426.5	19.00	18.50	145	150.84	5.40	-215		
18.04.2008	09:12	64.95	177	431	18.50	18.50	0	151.67	6.50	-265		
19.04.2008	10:59	90.73	175	433	18.50	18.50	0	152.04	6.50	-270		
21.04.2008	09:20	137.08	173	435	18.50	18.50	0	152.41	6.60	-290	1893	3760

Table A. 32 Raw data for 2.5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Starch (mgL^{-1})	TVFA (mgL^{-1})
15.04.2008	16:15	0.00	192	416	0.00	0.00	0	0	7.00	-155	17031	530
16.04.2008	09:18	17.05	192	416	11.00	11.00	214.60	69.36	3.70	-135		
16.04.2008	15:31	23.27	190	418	17.00	16.90	75	107.41	5.30			
17.04.2008	09:12	40.95	186.8	421.2	22.90	22.00	160	169.86	5.40	-205		
18.04.2008	09:09	64.90	183.3	424.7	22.00	22.00	0	170.63	6.50	-280		
19.04.2008	11:02	89.78	181.3	426.7	22.00	22.00	0	171.07	6.50	-256		
21.04.2008	09:23	137.13	179.3	428.7	22.00	22.00	0	171.51	6.50	-280	1938	3430

Table A. 33 Raw data for 3.5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Starch (mgL^{-1})	TVFA (mgL^{-1})
15.04.2008	16:15	0.00	192	416	0.00	0.00	0	0	7.00	-170	17975	530
16.04.2008	09:21	17.10	192	416	13.70	13.70	260	92.61	3.50	-120		
16.04.2008	15:34	23.31	189.5	418.5	14.30	14.10	20	98.32	5.20			
17.04.2008	09:16	41.05	187.1	420.9	17.90	17.60	60	125.39	6.10	-250		
18.04.2008	09:07	64.86	185.1	422.9	17.90	17.60	22	129.74	6.30	-275		
19.04.2008	11:05	90.86	183.1	424.9	17.90	17.60	0	131.37	6.30	-275		
21.04.2008	09:26	138.21	181.1	426.9	17.90	17.60	0	133.00	6.40	-290	2273	6280

Table A. 34 Raw data for 5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	Starch (mgL^{-1})	TVFA (mgL^{-1})
15.04.2008	16:15	0.00	192	416	0.00	0.00	0	0	7.00	-210	17285	530
16.04.2008	09:25	17.17	192	416	13.70	13.20	295	97.40	3.50	-115		
16.04.2008	15:38	23.40	189	419	14.70	14.50	20	107.08	5.50			
17.04.2008	09:19	41.09	187.6	420.4	19.50	18.80	38	135.66	6.30	-250		
18.04.2008	09:05	64.85	185.6	422.4	19.80	14.40	30	146.20	6.50	-280		
19.04.2008	11:07	90.85	183.6	424.4	14.40	14.40	0	146.48	6.50	-281		
21.04.2008	09:29	137.48	181.6	426.4	14.40	14.40	0	146.77	6.40	-280	2400	6530

A.1.2 Raw Data for Light Fermentative Hydrogen Production

A.1.2.1 Raw Data for Microbial Culture Selection

Table A. 35 Raw data for pure *Rhodobacter* sp.-RV

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
21.01.2008	17:00	0.00	245	65	0.00	0.00	0	0	7.45	-120	2340
22.01.2008	09:17	16.28	245	65	0.32	0.32	0	0.21			
23.01.2008	09:17	40.28	245	65	1.76	1.51	0	1.14			
24.01.2008	09:22	64.36	245	65	17.30	16.90	0	11.40			
25.01.2008	09:19	88.31	245	65	28.60	28.60	5	20.32	7.65	-355	
26.01.2008	10:52	113.87	235	75	31.00	30.00	0	22.12	7.30	-285	
28.01.2008	09:04	136.05	233	77	39.80	37.90	2	30.46	7.42	-308	
29.01.2008	09:19	184.30	231	79	38.80	37.80	0	31.18	7.50	-315	
30.01.2008	09:12	208.27	229	81	37.80	37.80	0	31.18	7.40	-291	
31.01.2008	10:10	233.23	227	83	37.80	37.80	0	31.18	7.30	-297	
01.02.2008	09:50	255.75	225	85	37.80	37.80	0	31.18	7.40	-293	1830

Table A. 36 Raw data for pure *Rhodobacter* sp.-NRLL

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
21.01.2008	17:00	0.00	245	65	0.00	0.00	0	0	7.45	-120	2680
22.01.2008	09:19	16.30	245	65	0.72	0.72	0	0.46			
23.01.2008	09:20	40.30	245	65	1.34	1.32	0	0.87			
24.01.2008	09:27	64.40	245	65	2.24	2.16	0	1.47			
25.01.2008	09:24	88.35	245	65	3.42	3.30	0	2.28	8.96	-230	
26.01.2008	10:57	113.90	236	74	12.40	12.10	0	9.02	7.50	-192	
28.01.2008	09:11	136.10	234	76	48.90	48.10	1	37.48	7.52	-268	
29.01.2008	09:23	184.30	232	78	58.50	57.90	4	47.93	7.50	-254	
30.01.2008	09:16	207.18	230	80	57.90	57.90	0	47.93	7.74	-308	
31.01.2008	10:15	232.17	225	85	57.90	57.90	0	47.93	7.70	-311	
01.02.2008	09:54	255.85	219	91	57.90	57.90	0	47.93	7.54	-326	1380

Table A. 37 Raw data for pure *Rhodobacter* sp.-DSMZ

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
21.01.2008	17:00	0.00	245	65	0.00	0.00	0	0	7.45	-120	2280
22.01.2008	09:24	16.40	245	65	0.30	0.30	0	0.19			
23.01.2008	09:24	40.40	245	65	0.35	0.35	0	0.22			
24.01.2008	09:31	64.50	245	65	1.29	1.19	0	0.83			
25.01.2008	09:27	88.45	245	65	2.78	2.69	0	1.87	9.21	-300	
26.01.2008	11:01	114.00	235	75	4.80	4.76	0	3.45	7.46	-270	
28.01.2008	09:15	136.25	233	77	4.76	4.76	0	3.45	8.85	-310	
29.01.2008	09:28	184.45	228	82	4.76	4.76	0	3.45	8.44	-310	
30.01.2008	09:20	208.35	222	88	4.76	4.76	0	3.45	8.00	-310	
31.01.2008	10:18	233.32	220	90	4.76	4.76	0	3.45	7.85	-301	
01.02.2008	09:57	256.97	215	95	4.76	4.76	0	3.45	7.60	-317	820

Table A. 38 Raw data for combination of RS-RV+RS-NRLL

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
12.02.2008	14:45	0.00	246	64	0.00	0.00	0	0	7.40	-140	2250
13.02.2008	09:39	18.90	246	64	0.31	0.32	0	0.19	7.50	-220	
14.02.2008	09:51	43.10	245	65	1.48	0.60	0	0.95	7.70	-310	
15.02.2008	09:05	66.28	242	68	2.70	2.70	0	2.40	7.90	-320	
16.02.2008	11:55	93.10	236	74	33.00	33.00	0	24.98	8.20	-331	
18.02.2008	09:10	138.28	231	79	33.00	33.00	0	26.63	8.80	-341	
19.02.2008	09:04	162.18	224	86	33.00	33.00	0	28.94	8.40	-340	
20.02.2008	09:07	186.23	220	90	33.00	33.00	0	30.26	8.00	-375	
21.02.2008	09:07	210.23	217	93	33.00	33.00	0	31.25	7.70	-354	
22.02.2008	09:02	234.21	214	96	33.00	33.00	0	32.24	7.70	-353	
23.02.2008	12:28	261.68	209	101	33.00	33.00	0	33.89	8.00	-365	
25.02.2008	08:57	306.16	203	107	33.00	33.00	0	35.87	7.40	-325	
26.02.2008	09:03	330.26	201	109	33.00	33.00	0	36.53	7.40	-334	810

Table A. 39 Raw data for combination of RS-RV+RS-DSMZ

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
12.02.2008	14:45	0.00	246	64	0.00	0.00	0	0	7.40	-136	1960
13.02.2008	09:43	18.97	246	64	0.30	0.30	0	0.19	7.50	-226	
14.02.2008	09:53	43.13	243	67	2.50	2.50	0	1.67	7.70	-325	
15.02.2008	09:08	66.38	240	70	3.20	3.20	0	2.24	7.70	-309	
16.02.2008	11:59	93.24	237	73	19.10	13.50	0.80	14.09	7.50	-330	
18.02.2008	09:14	138.49	235	75	35.50	35.50	7.40	33.49	7.40	-320	
19.02.2008	09:07	162.39	231	79	50.00	50.00	1.30	47.01	7.50	-312	
20.02.2008	09:10	186.44	229	81	57.80	42.80	0.10	54.39	7.50	-357	
21.02.2008	09:10	210.44	227	83	61.00	55.80	0	70.35	7.40	-333	
22.02.2008	09:05	234.39	225	85	63.40	58.00	0	77.93	7.40	-352	
23.02.2008	12:31	261.82	223	87	62.90	60.00	0	83.35	7.50	-358	
25.02.2008	09:00	306.30	221	89	64.20	60.00	0	88.29	7.40	-322	
26.02.2008	09:06	330.40	219	91	60.00	60.00	0	89.49	7.40	-330	1410

Table A. 40 Raw data for combination of RS-NRLL + RS-DSMZ

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
12.02.2008	14:45	0.00	246	64	0.00	0.00	0	0	7.40	-129	1970
13.02.2008	09:47	19.03	246	64	0.00	0.00	0	0	7.50	-204	
14.02.2008	09:57	43.19	243	67	1.95	1.95	0	1.30	7.70	-301	
15.02.2008	09:11	66.42	240	70	3.90	3.70	0	2.73	7.80	-317	
16.02.2008	12:04	93.38	235	75	3.70	3.70	0	2.91	8.20	-351	
18.02.2008	09:18	138.60	231	79	3.70	3.70	0	3.06	8.90	-332	
19.02.2008	09:12	162.50	225	85	3.70	3.70	0	3.28	8.50	-330	
20.02.2008	09:14	186.55	221	89	3.70	3.70	0	3.43	8.80	-365	
21.02.2008	09:14	210.55	217	93	3.70	3.70	0	3.58	7.90	-352	
22.02.2008	09:08	234.45	214	96	3.70	3.70	0	3.69	7.60	-349	
23.02.2008	12:34	261.92	211	99	3.70	3.70	0	3.80	7.50	-365	
25.02.2008	09:03	306.40	209	101	3.70	3.70	0	3.87	7.60	-322	
26.02.2008	09:10	330.50	207	103	3.70	3.70	0	3.95	7.50	-347	890

Table A. 41 Raw data for combination of RS-RV + RS-NRLL + RS-DSMZ

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP(mV)	TVFA (mgL^{-1})
12.02.2008	14:45	0.00	246	64	0.00	0.00	0	0	7.40	-124	1700
13.02.2008	09:51	19.10	246	64	6.90	6.90	0	4.41	7.50	-210	
14.02.2008	09:59	43.20	246	64	14.70	14.70	0	9.40	7.60	-400	
15.02.2008	Eyl.13	66.43	239	71	30.00	30.00	0	21.30	7.50	-316	
16.02.2008	12:07	93.33	236	74	55.70	55.70	2.90	42.08	7.40	-331	
18.02.2008	09:23	138.61	232	78	65.80	44.70	0.80	52.63	7.40	-312	
19.02.2008	09:18	162.51	229	81	59.80	57.00	2.70	67.98	7.50	-322	
20.02.2008	09:17	186.51	227	83	57.00	57.00	0	69.12	7.30	-314	
21.02.2008	09:17	210.51	225	85	57.00	57.00	0	70.26	7.30	-330	
22.02.2008	09:12	234.46	223	87	57.00	57.00	0	71.40	7.20	-359	
23.02.2008	12:41	261.94	221	89	57.00	57.00	0	72.54	7.30	-358	
25.02.2008	09:07	306.36	219	91	57.00	57.00	0	73.68	7.40	-327	
26.02.2008	09:14	330.46	217	93	57.00	57.00	0	74.82	7.60	-347	1260

A.1.2.2 Raw Data for Initial TVFA and NH4-N Concentrations

Table A. 42 Raw data for TVFAi=5795 mgL^{-1} and NH_4-N_i=183 mgL^{-1}

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	TOC (mgL^{-1})
08.05.2008	14:30	0.00	188	122	0.00	0.00	0	0	7.50	-137.5	5795	183	3890
09.05.2008	09:09	18.65	188	122	0.30	0.00	0	0.36	7.30	-252			
10.05.2008	09:14	42.68	187	123	0.30	0.00	0	0.73	7.10	-227			
12.05.2008	09:13	90.68	185	125	0.30	0.00	0	1.11	7.68	-240			
13.05.2008	09:10	114.68	182	128	0.30	0.00	0	1.49	7.70	-310			
14.05.2008	09:16	138.78	179	131	0.30	0.00	0	1.88	7.70	-350			
15.05.2008	09:40	163.18	176	134	0.36	0.36	0	2.36	7.74	-362			
16.05.2008	09:14	186.74	173	137	0.36	0.36	0	2.38	7.78	-378			
17.05.2008	12:00	213.51	170	140	0.36	0.36	0	2.39	7.40	-340			
20.05.2008	09:13	282.73	168	142	0.36	0.36	0	2.39	7.77	-362	4480	138	2722

Table A. 43 Raw data for $TVFA_i$=2930 mgL^{-1} and NH_4-N_i=73.5 mgL^{-1}

Date	**Clock**	**Time (h)**	**Vl(mL)**	**Vg (mL)**	**CH_2, i(%)**	**CH_2, i-1(%)**	**Vw(mL)**	**VH_2(mL)**	**pH**	**ORP**	**TVFA (mgL^{-1})**	**NH_4-N (mgL^{-1})**	**TOC (mgL^{-1})**
08.05.2008	14:30	0.00	188	122	0.00	0.00	0	0	7.50	-128	2930	73.50	1815
09.05.2008	09:13	18.70	188	122	0.00	0.00	0	0	7.34	-225			
10.05.2008	09:17	42.70	187	123	0.00	0.00	0	0	8.45	-190			
12.05.2008	09:16	90.70	183	127	0.30	0.30	0	0.38	9.20	-280			
13.05.2008	09:12	114.65	178	132	0.38	0.38	0	0.50	8.00	-285			
14.05.2008	09:18	138.75	175	135	0.38	0.38	0	0.51	8.40	-320			
15.05.2008	09:42	163.15	173	137	0.38	0.38	0	0.52	8.74	-325			
16.05.2008	09:16	186.75	170	140	0.38	0.38	0	0.53	8.70	-340			
17.05.2008	12:04	213.45	166	144	0.38	0.38	0	0.54	8.20	-330			
20.05.2008	09:16	282.71	162	148	0.38	0.38	0	0.56	8.50	-365	2450	10.50	811.50

Table A. 44 Raw data for TVFAi=2350 mgL^{-1} and NH_4-N_i=47 mgL^{-1}

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	TOC (mgL^{-1})
08.05.2008	14:30	0.00	188	122	0.00	0.00	0	0	7.50	-117	2350	47	1312.50
09.05.2008	09:15	18.75	188	122	0.00	0.00	0	0	7.30	-207			
10.05.2008	09:19	42.80	187	123	0.00	0.00	0	0	8.90	-190			
12.05.2008	09:19	90.80	183	127	4.00	3.90	0	5.08	9.20	-268			
13.05.2008	09:15	114.75	179	131	10.00	10.00	0	13.22	8.00	-245			
14.05.2008	09:21	138.85	176	134	23.50	23.50	0	31.61	7.40	-260			
15.05.2008	09:45	163.25	174	136	35.70	35.00	0.20	48.75	7.50	-280			
16.05.2008	09:19	186.81	172	138	43.00	41.80	1.20	61.00	7.90	-325			
17.05.2008	12:07	213.60	169	141	41.80	41.80	0	62.26	7.70	-310			
20.05.2008	09:19	282.81	166	144	41.80	41.80	0	63.51	7.60	-345	1250	8	847.25

Table A. 45 Raw data for $TVFA_i$=1490 mgL^{-1} and NH_4-N_i=33 mgL^{-1}

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	TOC (mgL^{-1})
08.05.2008	14:30	0.00	188	122	0.00	0.00	0	0	7.50	-103	1490	33	712.35
09.05.2008	09:22	18.86	188	122	0.00	0.00	2.90	0	7.20	-170			
10.05.2008	09:23	42.86	187	123	0.00	0.00	0	0	8.90	-208			
12.05.2008	09:25	90.86	184	126	16.90	16.70	0	21.29	9.00	-290			
13.05.2008	09:21	114.82	181	129	16.70	16.70	0	21.79	8.00	-245			
14.05.2008	09:28	138.92	178	132	16.70	16.70	0	22.29	7.40	-215			
15.05.2008	09:52	163.32	176	134	16.70	16.70	0	22.63	7.50	-193			
16.05.2008	09:26	186.89	174	136	16.70	16.70	0	22.96	7.50	-270			
17.05.2008	12:13	213.66	172	138	16.70	16.70	0	23.29	7.30	-177			
20.05.2008	09:24	282.86	170	140	16.70	16.70	0	23.63	7.30	-175	450	16	318.75

Table A. 46 Raw data for TVFAi=1200 mgL^{-1} and NH_4-N_i=18 mgL^{-1}

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	TOC (mgL^{-1})
08.05.2008	14:30	0.00	188	122	0.00	0.00	0	0	7.50	-100	1200	18	567.09
09.05.2008	09:28	18.96	188	122	0.00	0.00	2.90	0	7.20	-200			
10.05.2008	09:25	42.92	187	123	0.00	0.00	0	0	8.70	-212			
12.05.2008	09:30	90.92	181	129	0.96	0.95	0	1.23	8.60	-242			
13.05.2008	09:29	114.92	177	133	0.95	0.95	0	1.27	7.50	-200			
14.05.2008	09:34	139.00	175	135	0.95	0.95	0	1.29	7.20	-216			
15.05.2008	09:58	163.40	173	137	0.95	0.95	0	1.31	7.20	-200			
16.05.2008	09:31	186.85	171	139	0.95	0.95	0	1.33	7.20	-163			
17.05.2008	12:18	213.75	169	141	0.95	0.95	0	1.35	6.90	-172			
20.05.2008	09:30	282.95	167	143	0.95	0.95	0	1.37	6.90	-170	123	15	326.91

A.1.2.3 Raw Data for Initial Biomass Concentration

Table A. 47 Raw data for 0.5gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	-39	2340	41
06.03.2009	09:25	17.55	165	145	0.00	0.00	3.70	0	7.57	77.5		
07.03.2009	08:49	40.95	162	148	0.00	0.00	0	0	7.63	-1.1		
08.03.2009	13:20	69.46	158	152	0.32	0.32	0	0.48	7.64	-145.2		
09.03.2009	09:25	89.55	157	153	0.32	0.32	0	0.48	7.72	-104.2		
10.03.2009	09:34	113.70	152	158	5.81	5.81	0	9.17	7.96	-95.2		
11.03.2009	09:26	137.56	148	162	18.00	18.00	0	29.16	7.90	-92.6		
12.03.2009	09:16	161.39	145	165	23.90	23.90	0	39.43	7.81	-99		
13.03.2009	09:15	185.37	144	166	26.80	26.80	0	44.48	7.77	-128.4		
14.03.2009	17:27	217.54	140	170	25.30	25.30	0	44.48	7.80	-190.4		
16.03.2009	09:23	257.47	136	174	35.20	35.20	0	62.72	7.77	-80.2		
17.03.2009	09:26	281.50	132	178	39.20	39.20	0	71.25	7.68	-62.3	1084	0.2

Table A. 48 Raw data for 1gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	-110	2200	43
06.03.2009	09:27	17.51	165	145	0.00	0.00	3.10	0	7.57	75		
07.03.2009	08:51	40.89	162	148	0.00	0.00	0	0	7.67	-15.10		
08.03.2009	13:22	69.40	158	152	0.30	0.30	0	0.456	7.62	-150		
09.03.2009	09:27	89.48	157	153	0.30	0.30	0	0.459	7.68	-115		
10.03.2009	09:36	113.64	152	158	0.30	0.30	0	0.474	7.75	-106.40		
11.03.2009	09:28	137.49	148	162	4.65	4.65	0	7.533	7.98	-166.30		
12.03.2009	09:18	161.32	145	165	24.40	24.40	2.10	40.77	7.89	-149.30		
13.03.2009	09:17	185.30	144	166	34.20	34.20	4.80	58.92	7.88	-178.40		
14.03.2009	17:28	217.50	140	170	27.10	27.10	0	58.92	7.89	-209.10		
16.03.2009	09:24	257.43	136	174	18.10	18.10	0	58.92	8.00	-81.10		
17.03.2009	09:28	281.46	132	178	16.60	16.60	0	58.92	7.96	-57.10	1151	0.1

Table A. 49 Raw data for 1.5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	-149.10	2000	44
06.03.2009	09:31	17.48	165	145	0.00	0.00	5	0	7.57	37		
07.03.2009	08:53	40.84	162	148	0.30	0.30	0	0.44	7.68	-58.50		
08.03.2009	13:25	69.37	158	152	0.32	0.32	0	0.48	7.59	-163.30		
09.03.2009	09:30	89.45	157	153	0.30	0.30	0	0.48	7.71	-124.80		
10.03.2009	09:39	113.60	152	158	0.30	0.30	0	0.48	7.71	-134.30		
11.03.2009	09:30	137.45	148	162	0.39	0.39	0	0.64	7.95	-182.20		
12.03.2009	09:22	161.31	145	165	13.75	13.75	0	22.69	7.99	-149.50		
13.03.2009	09:22	185.31	144	166	14.50	14.50	0	24.08	7.96	-160.20		
14.03.2009	17:32	217.47	140	170	10.00	10.00	0	24.08	8.05	-189.40		
16.03.2009	09:29	257.42	136	174	5.28	5.28	0	24.08	8.26	-86.40		
17.03.2009	09:34	281.47	129	181	0.28	0.28	0	24.08	7.92	-70.11	910	0.30

Table A. 50 Raw data for 2.5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	-194.2	1940	45
06.03.2009	09:35	17.41	165	145	0.00	0.00	4.50	0	7.58	2		
07.03.2009	08:54	40.72	162	148	0.30	0.30	0	0.44	7.65	-107.5		
08.03.2009	13:27	69.72	158	152	0.32	0.32	0	0.48	7.58	-172.3		
09.03.2009	09:31	89.78	157	153	0.30	0.30	0	0.48	7.76	-154.2		
10.03.2009	09:41	113.94	152	158	0.00	0.00	0	0.48	7.60	-133.8		
11.03.2009	09:32	137.79	148	162	0.34	0.34	0	1.03	7.91	-243		
12.03.2009	09:23	161.64	145	165	1.58	1.58	0	3.09	7.95	-300		
13.03.2009	09:23	185.64	144	166	3.52	3.52	0	6.32	8.03	-312.5		
14.03.2009	17:33	217.80	140	170	3.56	3.56	0	6.53	8.10	-284.2		
16.03.2009	09:30	257.75	136	174	2.00	2.00	0	6.53	8.30	-297.8		
17.03.2009	09:31	281.75	131	179	0.18	0.18	0	6.53	8.13	-268	589	0.5

Table A. 51 Raw data for 3.5 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	-195	1880	44
06.03.2009	09:37	17.38	165	145	0.30	0.30	5.70	0.43	7.56	-88.5		
07.03.2009	08:56	40.69	162	148	0.34	0.34	0	0.50	7.68	-191.9		
08.03.2009	13:28	69.22	158	152	0.35	0.35	0	0.53	7.55	-179.7		
09.03.2009	09:32	89.29	157	153	0.34	0.34	0	0.53	7.74	-194.4		
10.03.2009	09:42	113.45	152	158	0.33	0.33	0	0.53	7.55	-150.3		
11.03.2009	09:33	137.30	148	162	0.35	0.35	0	0.57	7.72	-179.5		
12.03.2009	09:24	161.45	145	165	0.35	0.35	0	0.57	7.68	-220		
13.03.2009	09:24	185.45	144	166	0.45	0.45	0	0.74	7.76	-184.7		
14.03.2009	17:35	217.63	140	170	0.38	0.38	0	0.74	7.66	-213.1		
16.03.2009	09:31	257.56	136	174	0.37	0.37	0	0.74	7.63	-158.5		
17.03.2009	09:34	281.61	132	178	0.32	0.32	0	0.74	7.66	-159	1870	12.40

Table A. 52 Raw data for 3.5 gL^{-1} initial biomass concentration

Date	**Clock**	**Time (h)**	**Vl(mL)**	**Vg (mL)**	**CH_2, i(%)**	**CH_2, i-1(%)**	**Vw(mL)**	**VH_2(mL)**	**pH**	**ORP (mV)**	**TVFA (mgL^{-1})**	**NH_4-N (mgL^{-1})**
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	12	2610	46
06.03.2009	09:39	17.35	165	145	0.00	0.00	0	0	7.58	-177.20		
07.03.2009	08:57	40.65	162	148	0.31	0.31	0	0.45	7.61	-150.20		
08.03.2009	13:31	69.21	158	152	0.00	0.00	0	0.45	7.52	-63.70		
09.03.2009	09:34	89.26	157	153	0.31	0.31	0	0.93	7.68	-165.10		
10.03.2009	09:44	113.42	152	158	0.32	0.32	0	0.96	7.49	-172.90		
11.03.2009	09:35	137.27	148	162	0.33	0.33	0	0.99	7.65	-168.30		
12.03.2009	09:26	161.12	145	165	0.35	0.35	0	1.03	7.62	-195.70		
13.03.2009	09:26	185.12	144	166	0.35	0.35	0	1.03	7.60	-183.40		
14.03.2009	17:36	217.28	140	170	0.35	0.35	0	1.03	7.59	-192.40		
16.03.2009	09:32	257.21	136	174	0.36	0.36	0	1.06	7.55	-148.10		
17.03.2009	09:39	281.32	132	178	0.33	0.33	0	1.06	7.63	-150	1877	13.80

Table A. 53 Raw data for control experiment without any microbial inoculation

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})
05.03.2009	15:00	0.00	165	145	0.00	0.00	0	0	7.48	-8.40	2200	43
06.03.2009	09:25	17.58	165	145	0.00	0.00	3.60	0	7.49	110		
07.03.2009	08:49	40.98	162	148	0.00	0.00	0	0	7.48	24.20		
08.03.2009	13:20	69.49	158	152	0.30	0.30	0	0.45	7.48	-114.50		
09.03.2009	09:25	89.56	157	153	0.31	0.31	0	0.47	7.50	-29		
10.03.2009	09:34	113.71	152	158	0.31	0.31	0	0.48	7.46	11.20		
11.03.2009	09:26	137.57	148	162	0.32	0.32	0	0.51	7.48	12.30		
12.03.2009	09:16	161.40	145	165	0.42	0.42	0	0.51	7.47	1.90		
13.03.2009	09:15	185.38	144	166	0.42	0.42	0	0.51	7.47	-53.40		
14.03.2009	17:27	217.58	140	170	0.42	0.42	0	0.51	7.53	-169.50		
16.03.2009	09:23	257.51	136	174	0.42	0.42	0	0.51	7.53	-15.10		
17.03.2009	09:25	281.54	132	178	0.42	0.42	0	0.51	7.59	37.20	1312	14

A.1.2.4 Raw Data for Light Source and Light Intensity Experiments

Table A. 54 Raw data for tungsten & infrared light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	16:00	0.00	250	60	0.00	0.00	0	0	7.30	-255	2100	347.45
13.06.2009	12:11	20.18	250	60	13.30	13.30	4.30	8.55	7.26	-200		
14.06.2009	12:28	44.46	246	64	56.10	56.10	12.60	43.55	7.17	-120.40		
15.06.2009	09:46	65.76	241	69	72.50	72.50	12.80	66.94	7.19	-121.20		
16.06.2009	09:46	89.76	236	74	77.70	77.70	21.10	90.81	7.10	-147		
17.06.2009	09:30	113.49	231	79	87.00	87.00	10.90	111.52	7.06	-104.30		
18.06.2009	09:39	137.64	226	84	87.10	87.10	6	121.18	7.03	-126.10		
19.06.2009	09:21	161.34	221	89	85.70	85.70	1.40	122.38	6.97	-150.50		
22.06.2009	09:20	185.32	216	94	41.00	41.00	0	122.38	7.19	-235.20	1382	

Table A. 55 Raw data for tungsten light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	16:00	0.00	250	60	0.00	0.00	0	0	7.30	-255	2100	347.45
13.06.2009	12:08	20.13	250	60	13.80	13.80	4.60	8.91	7.23	-188		
14.06.2009	12:25	44.41	246	64	57.30	57.30	10.80	43.42	7.16	-135		
15.06.2009	09:43	65.71	241	69	71.80	71.80	11	64.26	7.20	-113.90		
16.06.2009	09:36	89.59	236	74	80.00	80.00	9.20	81.28	6.98	-123.30		
17.06.2009	09:26	113.42	231	79	81.20	81.20	10	94.34	7.03	-103.50		
18.06.2009	09:37	137.60	226	84	83.20	83.20	5.60	104.74	6.89	-114		
19.06.2009	09:20	161.32	221	89	79.90	79.90	0	104.74	6.63	-129.60		
22.06.2009	09:10	185.30	216	94	55.00	55.00	0	104.74	7.02	-128.90	1413	

Table A. 56 Raw data for fluorescent light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	16:00	0.00	250	60	0.00	0.00	0	0	7.30	-255	2100	347.45
13.06.2009	12:13	20.22	250	60	25.60	25.60	8.30	17.48	7.31	-216		
14.06.2009	12:31	44.52	246	64	63.00	63.00	17.90	53.72	7.25	-119.20		
15.06.2009	09:49	65.82	241	69	78.80	78.80	11.40	76.75	7.29	-114.20		
16.06.2009	09:48	89.80	236	74	78.80	78.80	22	94.09	7.22	-128.30		
17.06.2009	09:33	113.55	231	79	81.60	81.60	2.70	102.44	7.25	-110		
18.06.2009	09:40	137.66	226	84	70.00	70.00	0	102.44	7.16	-124.40		
19.06.2009	09:23	161.38	221	89	51.00	51.00	0	102.44	7.22	-140		
22.06.2009	09:22	185.36	216	94	16.30	16.30	0	102.44	7.35	-277.20	1014	

Table A. 57 Raw data for infrared light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	16:00	0.00	250	60	0.00	0.00	0	0	7.30	-255	2100	347.45
13.06.2009	12:16	20.26	250	60	1.58	1.58	1.20	0.96	7.23	-176		
14.06.2009	12:34	44.56	246	64	6.20	6.20	0	3.98	7.18	-115		
15.06.2009	09:51	65.85	241	69	11.80	11.80	0	8.16	7.18	-112.50		
16.06.2009	09:52	89.86	236	74	13.00	13.00	0	9.63	7.16	-120		
17.06.2009	09:42	113.69	231	79	8.43	8.43	0	9.63	7.09	-100		
18.06.2009	09:43	137.71	226	84	9.10	9.10	0	10.62	6.93	-105		
19.06.2009	09:26	161.43	221	89	5.09	5.09	0	10.62	7.06	-188.20		
22.06.2009	09:25	185.41	216	94	0.64	0.64	0	10.62	7.16	-342.40	1926	

Table A. 58 Raw data for halogen light source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	Glucose (mgL^{-1})	NH_4-N (mgL^{-1})
12.06.2009	16:00	0.00	250	60	0.00	0.00	0	0	7.30	-255	2100	347.45	4.00
13.06.2009	12:18	20.30	250	60	24.30	24.30	9.80	16.96	7.30	-205.20			
14.06.2009	12:37	44.61	246	64	64.50	64.50	35.40	66.49	7.25	-118.10	1243	241.30	0.20
15.06.2009	09:53	65.87	241	69	78.50	78.50	40	110.77	7.22	-132			
16.06.2009	09:53	89.87	236	74	86.50	86.50	42	156.95	7.19	-143.30	1163	130.94	0.60
17.06.2009	09:59	113.97	231	79	88.30	88.30	30	189.19	7.19	-115			
18.06.2009	09:46	137.80	226	84	88.80	88.80	20	211.78	7.22	-115.20	987	144.40	0.50
19.06.2009	09:29	161.52	221	89	92.50	92.50	8	226.91	7.14	-146			
22.06.2009	09:27	185.48	216	94	96.50	96.50	10.90	245.82	7.37	-180	842	113.35	0.40

Table A. 59 Raw data for sun light

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	16:00	0.00	167	143	0.00	0.00	0	0	7.30	-255	2100	347.45
13.06.2009	11:43	19.71	167	143	0.49	0.49	0	0.70	7.25	-200		
14.06.2009	13:03	45.04	163	147	2.78	2.78	0.60	4.10	7.23	-120.50		
15.06.2009	10:16	66.25	158	152	16.50	16.50	3.80	25.72	7.21	-130		
16.06.2009	10:15	90.23	153	157	27.50	27.50	8.60	46.18	7.23	-144		
17.06.2009	10:07	114.09	148	162	34.20	34.20	8.20	61.21	7.22	-106.80		
18.06.2009	10:04	138.04	143	167	37.50	37.50	6.60	70.91	7.17	-117.90		
19.06.2009	09:45	161.72	138	172	37.50	37.50	0.20	72.86	7.18	-145		
22.06.2009	09:43	185.68	133	177	23.60	23.60	0	72.86	7.19	-363.50	1159	

Table A. 60 Raw data for 1 klux halogen lamp light source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.16	89.72
02.07.2009	09:39	18.85	200	110	0.40	0.40	2.50	0.45	7.16	-155			
03.07.2009	09:27	42.42	196	114	0.66	0.66	0	0.76	7.44	-142			
04.07.2009	10:16	67.29	192	118	1.64	1.64	0	1.94	7.58	-182.20			
06.07.2009	09:07	114.44	188	122	1.92	1.92	0	2.35	7.25	-272.80			
07.07.2009	10:31	139.84	184	126	2.40	2.40	0	3.03	7.81	-411.10			
08.07.2009	09:20	163.02	183	127	7.50	7.50	0	3.03	7.43	-373.30			
09.07.2009	09:49	187.50	179	131	0.96	0.96	0	3.03	8.20	-478.10	1114		82.11

Table A. 61 Raw data for 3 klux halogen lamp light source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.167	89.72
02.07.2009	09:40	18.83	200	110	0.68	0.68	6.10	0.78	7.36	-150			
03.07.2009	09:31	42.68	196	114	5.58	5.58	1	6.45	7.60	-165			
04.07.2009	10:18	67.46	192	118	21.20	21.20	9.80	27.19	7.63	-183			
06.07.2009	09:11	114.57	188	122	40.00	40.00	22.6	60.01	7.47	-295.90			
07.07.2009	10:34	139.95	184	126	43.80	43.80	5.10	68.63	7.65	-375.20			
08.07.2009	09:24	162.78	183	127	39.00	39.00	0	68.63	7.60	-397.80			
09.07.2009	09:50	187.21	179	131	24.70	24.70	0	68.63	7.70	-558	1007		78.37

Table A. 62 Raw data for 5 klux halogen lamp light source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.16	89.72
02.07.2009	09:42	18.80	200	110	0.70	0.70	4.70	0.80	7.45	-150			
03.07.2009	09:35	42.68	196	114	14.20	14.20	8	17.35	7.62	-160	1321	1.67	87.40
04.07.2009	10:20	67.26	192	118	32.20	32.20	13.20	43.41	7.61	-181			
06.07.2009	09:13	114.14	188	122	47.00	47.00	38	80.61	7.45	-275.60	1084	0.66	86.10
07.07.2009	10:35	139.51	184	126	48.40	48.40	3.40	85.90	7.54	-326.30			
08.07.2009	09:23	162.71	183	127	44.30	44.30	0	85.90	7.53	-368.90			
09.07.2009	09:52	187.19	179	131	33.00	33.00	0	85.90	7.41	-519.60	1051	0.33	69.02

Table A. 63 Raw data for 7 klux halogen lamp light source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.16	89.72
02.07.2009	09:44	18.76	200	110	0.63	0.60	5.20	0.72	7.48	-140			
03.07.2009	09:39	42.67	196	114	13.60	13.60	4.20	16.10	7.60	-155			
04.07.2009	10:22	67.38	192	118	31.00	31.00	14.60	41.70	7.58	-173			
06.07.2009	09:15	114.49	188	122	43.00	43.00	22.10	67.09	7.45	-284.40			
07.07.2009	10:37	139.12	184	126	41.00	41.00	0.40	67.25	7.51	-336.20			
08.07.2009	09:27	162.12	183	127	30.00	30.00	0	67.25	7.51	-381.50			
09.07.2009	09:54	186.57	179	131	17.00	17.00	0	67.25	7.46	-505.30	1075		68.27

Table A. 64 Raw data for 10 klux halogen lamp light source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.16	89.72
02.07.2009	09:46	18.73	200	110	0.76	0.76	8	0.89	7.40	-135			
03.07.2009	09:43	42.68	196	114	1.57	1.57	0	1.85	7.46	-119.40			
04.07.2009	10:23	67.51	192	118	2.45	2.45	0	2.95	7.61	-147.70			
06.07.2009	09:17	114.41	188	122	3.00	3.00	0	3.72	7.65	-402.10			
07.07.2009	10:38	139.76	184	126	6.00	6.00	0	7.62	7.77	-427.20			
08.07.2009	09:35	162.71	183	127	0.89	0.89	0	7.62	7.83	-434.20			
09.07.2009	09:57	187.07	179	131	0.32	0.32	0	7.62	8.00	-538.30	1097		86.35

Table A. 65 Raw data for sun light

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.16	89.72
02.07.2009	09:18	19.20	200	110	0.33	0.33	0	0.36	7.41	-128.30			
03.07.2009	09:10	43.06	196	114	0.69	0.69	0	0.78	7.49	-136.50			
04.07.2009	09:58	67.86	192	118	2.69	2.69	0	3.17	7.44	-155.40			
06.07.2009	09:37	115.51	188	122	9.56	9.56	0	11.66	7.60	-300.20			
07.07.2009	10:19	140.64	184	126	7.14	7.14	0	11.66	7.80	-408.70			
08.07.2009	09:43	163.87	183	127	4.20	4.20	0	11.66	7.96	-417.10			
09.07.2009	10:02	188.18	179	131	2.18	2.18	0	11.66	7.72	-507.30	1176		68.65

Table A. 66 Raw data for control bottle of the experiment for investigating the light intensity of halogen lamp source

Date	Clock	Time (h)	Vl(mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	16:30	0.00	200	110	0.00	0.00	0	0	7.30	-92	1465	2.16	89.72
02.07.2009	09:21	19.15	200	110	0.00	0.00	0	0					
03.07.2009	09:13	43.02	200	110	0.00	0.00	0	0	7.64	-124.20			
04.07.2009	10:01	67.82	196	114	1.17	1.17	0	1.33					
06.07.2009	09:07	114.92	196	114	0.46	0.46	0	1.33	7.65	-150.20			
07.07.2009	10:40	140.47	192	118	1.10	1.10	0	2.10					
08.07.2009	09:36	163.40	192	118	0.91	0.91	0	2.10	7.81	-414.80			
09.07.2009	09:57	188.34	188	122	0.78	0.78	0	2.10	7.55	-470.20	1465		76.5

A.1.3 Raw Data for Combined Fermentative Hydrogen Production

A.1.3.1 Raw Data for the Effects of Dark to Light Biomass Ratio

Table A. 67 Raw data for D/L of 1/2

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
04.11.2008	22:00	0.00	133	177	0.00	0.00	0	0	7.30	66	165	10.40	4782.20
05.11.2008	09:28	11.46	133	177	0.99	0.99	7.20	1.82	6.33	-77			
06.11.2008	09:29	35.47	119	191	0.99	0.99	0	1.96	6.37	-63			
07.11.2008	09:12	59.20	112	198	2.60	2.60	0	5.21	6.56	-52			
08.11.2008	13:10	87.17	97	213	3.92	3.92	0	8.42	6.65	-105			
10.11.2008	09:35	131.60	90	220	3.92	3.92	0	8.69	6.84	-100			
11.11.2008	10:05	156.18	84	226	6.00	6.00	0	13.63	6.80	-45			
12.11.2008	10:54	180.91	73	237	8.52	8.52	0	20.26	6.90	-105			
13.11.2008	09:53	203.89	65	245	8.52	8.52	0	20.94	7.12	-195			
14.11.2008	10:10	228.17	61	249	8.52	8.52	0	21.28	7.27	-91			
17.11.2008	09:42	299.7	56	254	8.52	8.52	0	21.71	7.27	-75	95	5.60	793.60

Table A. 68 Raw data for D/L of 1/4

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
04.11.2008	22:00	0.00	133	177	0.00	0.00	0	0	7.30	60	129	10.40	4029.30
05.11.2008	09:30	11.50	133	177	3.77	3.77	1.20	6.68	6.54	-110			
06.11.2008	09:32	35.53	112	198	3.88	3.88	3	7.80	6.38	-115			
07.11.2008	09:19	59.31	105.5	204.5	3.88	3.88	0	8.05	6.58	-133			
08.11.2008	13:12	87.19	95	215	3.88	3.88	0	8.46	6.66	-135			
10.11.2008	09:36	131.59	89	221	10.00	10.00	3	22.52	6.89	-124			
11.11.2008	10:02	156.02	84	226	20.00	20.00	5.30	46.68	6.90	-70			
12.11.2008	11:00	180.02	76	234	20.00	20.00	7.10	49.70	6.90	-112			
13.11.2008	09:56	202.95	71	239	20.00	20.00	0	50.70	7.13	-235			
14.11.2008	10:11	227.2	66	244	20.00	20.00	0	51.70	7.23	-161			
17.11.2008	09:44	298.75	63	247	20.00	20.00	0	52.30	7.18	-62	92	7.20	793.16

Table A. 69 Raw data for D/L of 1/7

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
04.11.2008	22:00	0.00	133	177	0.00	0.00	0	0	7.30	54.4	151	11.20	4115.10
05.11.2008	09:36	11.60	133	177	1.31	1.31	10.10	2.45	6.10	8			
06.11.2008	09:42	35.70	126	184	3.40	3.40	13.60	6.85	7.00	-76			
07.11.2008	09:37	59.61	117	193	3.69	3.69	0	7.71	6.90	-275			
08.11.2008	13:16	87.25	100	210	3.69	3.69	0	8.34	7.00	-230			
10.11.2008	09:41	131.67	95	215	10.00	10.00	3	22.39	7.20	-269			
11.11.2008	09:55	155.90	89	221	31.00	31.00	5.30	71.04	7.20	-235			
12.11.2008	11:10	181.15	81	229	31.00	31.00	0	73.52	7.30	-250			
13.11.2008	10:02	204.01	74	236	31.00	31.00	0	75.69	7.50	-176			
14.11.2008	10:15	228.92	68	242	31.00	31.00	0	77.55	7.60	-365			
17.11.2008	09:47	299.75	68	242	31.00	31.00	0	77.55	7.50	-131	86	5.60	807.79

Table A. 70 Raw data for D/L of 1/10

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
04.11.2008	22:00	0.00	133	177	0.00	0.00	0	0	7.30	51.40	110	10.40	4636.50
05.11.2008	09:39	11.65	133	177	1.48	1.48	10	2.91	6.18	0			
06.11.2008	09:50	35.83	124	186	3.00	3.00	8.50	6.25	6.70	-155			
07.11.2008	09:25	59.41	117	193	4.47	4.47	0	9.30	6.90	-270			
08.11.2008	13:18	87.29	100	210	8.53	8.53	0	18.59	7.13	-225			
10.11.2008	09:42	131.69	94	216	26.90	26.90	19.90	61.94	7.27	-238			
11.11.2008	09:51	155.84	89	221	26.90	26.90	0	63.29	7.28	-286			
12.11.2008	11:13	181.21	81	229	26.90	26.90	0	65.44	7.42	-228.80			
13.11.2008	10:04	204.06	76	234	26.90	26.90	0	66.79	7.60	-218			
14.11.2008	10:16	228.26	65	245	26.90	26.90	0	69.74	7.77	-235			
17.11.2008	09:48	299.79	62	248	26.90	26.90	0	70.55	7.80	-122	103	3.20	814.30

Table A. 71 Raw data for only dark bacterial culture inoculation

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH_2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH_2 (mL)**	**pH**	**ORP(mV)**	**TVFA (mgL^{-1})**	**NH_4-N (mgL^{-1})**	**Starch (mgL^{-1})**
04.11.2008	22:00	0.00	133	177	0.00	0.00	0	0	7.30	75	207	4	4224.05
05.11.2008	09:42	11.70	133	177	1.28	1.28	7.20	2.35	6.70	5			
06.11.2008	09:53	35.88	126	184	4.28	4.28	8	8.30	6.30	-130			
07.11.2008	09:30	59.49	109	201	6.00	6.00	0	12.45	7.00	-350			
08.11.2008	13:20	87.32	96	214	6.00	6.00	0	13.27	7.00	-340			
10.11.2008	09:57	131.94	90	220	6.00	6.00	0	13.63	7.20	-350			
11.11.2008	09:48	155.79	82	228	10.00	10.00	0	23.23	7.20	-339			
12.11.2008	11:17	181.27	74	236	23.20	23.20	5.20	56.39	7.20	-310			
13.11.2008	10:07	205.10	71	239	35.60	35.60	10.70	90.53	7.20	-315			
14.11.2008	10:19	229.3	67	243	35.60	35.60	0	91.95	7.30	-300			
17.11.2008	09:50	300.82	64	246	35.60	35.60	0	93.02	7.40	-330	521	4	730.85

Table A. 72 Raw data for only light bacterial culture inoculation

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
04.11.2008	22:00	0.00	133	177	0.00	0.00	0	0	7.30	50	101	12	4664.71
05.11.2008	09:42	11.73	133	177	0.64	0.64	6.80	1.17	6.74	-25			
06.11.2008	10:00	35.99	126	184	8.00	8.00	23.50	16.64	6.34	-235			
07.11.2008	09:34	59.55	126	184	9.49	9.49	0	19.38	7.00	-302			
08.11.2008	13:21	87.33	98	212	9.49	9.49	0	22.04	7.14	-287.40			
10.11.2008	09:59	131.98	95	215	13.50	13.50	0	30.94	7.22	-295			
11.11.2008	09:43	155.71	90	220	20.00	20.00	5.30	46.98	7.12	0			
12.11.2008	11:23	181.38	83	227	37.20	37.20	4.40	89.06	7.19	-279			
13.11.2008	10:09	204.15	75	235	37.20	37.20	0	92.04	7.32	-111			
14.11.2008	10:22	228.362	70	240	37.20	37.20	0	93.90	7.45	-280			
17.11.2008	09:54	299.89	67	243	37.20	37.20	0	95.01	7.55	-250	163	5.60	882.15

A.1.3.2 Raw Data for Initial Substrate and Biomass Concentrations

Table A. 73 Raw data for 2.5 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
28.08.2008	14:15	0.00	179	131	0.00	0.00	0	0	7.50	51	83	2.20	2193
29.08.2008	09:28	19.22	179	131	8.77	8.77	6.30	12.04	7.20	-245			
30.08.2008	10:10	43.92	177	133	11.81	10.80	0	16.25	7.00	-320			
01.09.2008	09:30	91.25	163	147	10.80	10.80	0	17.77	7.50	-362			
02.09.2008	09:28	115.20	154	156	10.80	10.80	0	18.74	7.50	-365			
03.09.2008	09:47	139.52	150	160	10.80	10.80	0	19.17	7.46	-355	439		511

Table A. 74 Raw data for 5 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
28.08.2008	14:15	0.00	179	131	0.00	0.00	0	0	7.5	75	166	3.30	3767
29.08.2008	09:31	19.27	179	131	7.63	6.73	6.20	10.41	7.27	-256			
30.08.2008	10:13	43.97	176	134	17.80	17.40	0.30	25.50	6.67	-260			
01.09.2008	09:32	91.28	170	140	17.40	17.40	0	26.54	7.29	-371			
02.09.2008	09:31	115.28	161	149	17.40	17.40	0	28.11	7.27	-375			
03.09.2008	09:49	139.58	157	153	17.40	17.40	0	28.80	7.22	-370	1300		1129

Table A. 75 Raw data for 7.5 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
28.08.2008	14:15	0.00	179	131	0.00	0.00	0	0	7.5	72	129	4.80	5846
29.08.2008	09:36	19.35	179	131	9.12	9.12	8.60	12.52	6.92	-272			
30.08.2008	10:18	44.05	176	134	20.00	20.00	24.50	31.73	6.35	-315			
01.09.2008	09:36	91.35	166	144	20.00	20.00	0	33.73	7.2	-370			
02.09.2008	09:34	115.30	157	153	20.00	20.00	0	35.53	7.18	-339			
03.09.2008	09:49	139.58	153	157	20.00	20.00	0	36.33	7.1	-340	2150		1467

Table A. 76 Raw data for 10 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
28.08.2008	14:15	0.00	179	131	0.00	0.00	0	0	7.50	62	157	5.60	8183
29.08.2008	09:38	19.38	179	131	9.39	9.39	9.60	13.20	6.80	-270		4.20	
30.08.2008	10:20	44.08	176	134	22.60	21.80	40	39.90	6.15	-312		2.20	
01.09.2008	09:38	91.38	169	141	21.80	21.80	0	41.43	7.14	-370		8	
02.09.2008	09:37	115.28	160	150	21.80	21.80	0	43.39	7.08	-372		14	
03.09.2008	09:55	139.58	156	154	21.80	21.80	0	44.26	7.01	-362	3370	17.50	1782

Table A. 77 Raw data for 15 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
28.08.2008	14:15	0.00	179	131	0.00	0.00	0	0	7.50	62	212	7.60	12048
29.08.2008	09:42	19.45	179	131	14.00	14.00	25	21.84	6.60	-245			
30.08.2008	10:24	44.15	176	134	38.20	35.90	7.10	57.40	5.60	-260			
01.09.2008	09:41	91.43	167	143	35.90	35.90	3.90	61.80	6.70	-320			
02.09.2008	09:39	115.38	157	153	35.90	35.90	0	65.39	6.90	-335			
03.09.2008	09:58	139.68	153	157	35.90	35.90	0	66.82	6.80	-332	4870		2190

Table A. 78 Raw data for 20 gL^{-1} initial WP concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
28.08.2008	14:15	0.00	179	131	0.00	0.00	0	7.50	52	246	7.60	15570	4250.15
29.08.2008	09:46	19.52	179	131	19.00	18.90	39	7.00	-207	1365	18	11982	1370.76
30.08.2008	10:28	44.22	174	136	38.80	37.00	148.80	5.00	-209	4070	11	6878	1539.12
01.09.2008	09:44	91.48	164	146	37.00	37.00	35.30	7.00	-330	6400	57	3100	348.17
02.09.2008	09:42	115.43	153	157	37.00	37.00	0	7.00	-335	7070	105	2193	211.07
03.09.2008	10:01	139.73	149	161	37.00	37.00	0	7.00	-355	7060	120	2029	195.57

Table A. 79 Raw data for control bottle without any microbial inoculation

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	50	97	44	4046
27.11.2008	09:34	14.07	237.5	72.5	0.00	0.00	1.10	0	7.20	42.50			
28.11.2008	10:20	38.83	226	84	0.58	0.58	0	0.48	7.10	50			
29.11.2008	13:05	65.58	222	88	24.50	24.50	8.60	23.66	6.10	91			
01.12.2008	09:43	110.22	211	99	36.60	36.60	9.80	41.92	6.52	-58.60			
02.12.2008	09:35	134.08	202	108	36.60	36.60	0	41.92	6.40	-4.80			
03.12.2008	12:14	160.72	195	115	36.60	36.60	0	41.92	6.72	0			
04.12.2008	09:19	181.80	184	126	36.60	36.60	0	41.92	6.55	40	1426	30	449.77

Table A. 80 Raw data for 0.5gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP(mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	63	8	37	3921
27.11.2008	09:39	14.15	237.5	72.5	10.00	10.00	11.40	8.39	5.63	45			
28.11.2008	09:29	37.98	226	84	34.00	34.00	9.40	32.89	6.79	-15			
29.11.2008	13:10	65.65	212	98	49.80	49.80	10.30	58.26	7.11	35			
01.12.2008	09:45	110.23	204	106	42.30	42.30	0	58.26	7.27	-102.60			
02.12.2008	09:38	134.11	199	111	15.50	15.50	0	58.26	7.18	-11			
03.12.2008	12:17	160.75	192	118	11.40	11.40	0	58.26	7.34	-77			
04.12.2008	09:20	181.80	177	133	0.00	0.00	0	58.26	7.29	0	1758	3	624.19

Table A. 81 Raw data for 1.1gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	57	142	43	3921	353
27.11.2008	09:48	14.30	237.5	72.5	7.00	7.00	6.40	5.52	6.02	-25	1357	9.60	2322	635
28.11.2008	09:37	38.11	223	87	32.50	32.50	20.90	35.51	6.65	-6	1751	11.20	961	106
29.11.2008	13:15	65.74	215	95	35.50	35.50	3.80	42.31	6.90	20	1743	4	831	71
01.12.2008	09:55	110.40	206	104	68.00	68.00	57.50	118.40	7.02	-138.2	2126	2.40	828	79
02.12.2008	09:40	134.15	199	111	40.00	40.00	0	118.40	7.00	-40				
03.12.2008	12:18	160.78	190	120	24.20	24.20	0	118.40	7.30	-115	1872	0.80	980	90
04.12.2008	09:21	181.23	177	133	13.50	13.50	0	118.40	7.21	-50	1523	5	740	85

Table A. 82 Raw data for 1.5gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	54	94	40	4058
27.11.2008	09:51	14.35	237.5	72.5	5.42	5.42	6.80	4.29	6.21	-23			
28.11.2008	09:45	38.25	227	83	33.50	33.50	24.90	36.51	6.56	-19			
29.11.2008	13:18	65.80	215	95	33.60	33.60	4.30	42.07	6.67	-60			
01.12.2008	10:08	110.63	204	106	12.70	12.70	0	42.07	7.00	-233.10			
02.12.2008	09:42	134.36	199	111	8.18	8.18	0	42.07	6.90	-150			
03.12.2008	12:20	160.99	190	120	2.32	2.32	0	42.07	7.10	-260			
04.12.2008	09:23	182.04	174	136	0.00	0.00	0	42.07	7.18	-240	1732	8	451.74

Table A. 83 Raw data for 2.5gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	49	154	39	3987
27.11.2008	09:55	14.42	237.5	72.5	7.90	7.90	11.50	6.63	6.01	-51			
28.11.2008	09:52	38.36	227	83	31.20	31.20	17.90	32.38	6.65	-115			
29.11.2008	13:21	65.85	219	91	28.40	28.40	0	32.38	6.90	-220			
01.12.2008	10:12	110.70	211	99	11.50	11.50	0	32.38	7.54	-222			
02.12.2008	09:46	134.26	206	104	3.27	3.27	0	32.38	7.55	-130			
03.12.2008	12:22	160.72	196	114	1.38	1.38	0	32.38	7.85	-250			
04.12.2008	09:39	181.75	178	132	0.00	0.00	0	32.38	7.69	-352	596	10	856.77

Table A. 84 Raw data for 3.5gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	44	155	43	3904
27.11.2008	09:58	14.47	237.5	72.5	5.69	5.69	9.30	4.65	6.38	-27			
28.11.2008	10:02	38.53	227	83	27.80	27.80	13.30	27.30	6.50	-63			
29.11.2008	13:24	65.90	219	91	27.20	27.20	5.40	28.77	6.68	-325			
01.12.2008	10:15	110.05	210	100	11.00	11.00	0	28.76	7.16	-380.50			
02.12.2008	09:48	133.60	206	104	6.00	6.00	0	28.76	7.17	-363			
03.12.2008	12:24	160.20	197	113	2.88	2.88	0	28.76	7.68	-403.60			
04.12.2008	09:45	181.25	181	129	0.00	0.00	0	28.76	7.70	-400	730	9	1034.26

Table A. 85 Raw data for 5gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw(mL)	VH_2(mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})
26.11.2008	19:30	0.00	237.5	72.5	0.00	0.00	0	0	7.30	43	215	29	4149
27.11.2008	10:01	14.52	237.5	72.5	9.48	9.48	15.20	8.31	5.98	-38			
28.11.2008	10:06	38.60	227	83	25.00	25.00	7.70	24.11	6.89	-92			
29.11.2008	13:27	65.95	222	88	16.70	16.70	0.90	24.26	6.89	-345			
01.12.2008	10:18	110.80	212	98	5.20	5.20	0	24.26	7.46	-362.20			
02.12.2008	09:49	134.31	206	104	0.30	0.30	0	24.26	7.68	-330			
03.12.2008	12:25	160.90	200	110	0.30	0.30	0	24.26	8.00	-400			
04.12.2008	09:31	181.81	181	129	0.00	0.00	0	24.26	7.78	-392	395	3	1129.12

A.1.3.3 Raw Data for Light Source, Light Intensity and Lighting Regime

Table A. 86 Raw data for tungsten & infrared light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	12:00	0.00	200	110	0.00	0.00	0	0	7.00	-125	566	6.50	4260.70	4238.47
13.06.2009	11:48	23.80	200	110	37.20	37.20	228	125.73	5.95	-46.5				
14.06.2009	12:47	48.78	194	116	13.90	13.90	0	125.73	6.83	-177.40				
15.06.2009	09:58	69.96	189	121	15.30	15.30	0	128.12	6.92	-148.60				
16.06.2009	09:56	93.93	184	126	10.00	10.00	0	128.12	6.89	-160				
17.06.2009	09:45	117.74	179	131	10.00	10.00	0	128.12	6.86	-116.40				
18.06.2009	09:50	141.82	174	136	6.55	6.55	0	128.12	6.86	-195.10				
19.06.2009	09:32	165.52	169	141	4.56	4.56	0	128.12	6.87	-220.20	1293		188.75	70.89

Table A. 87 Raw data for tungsten light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	12:00	0.00	200	110	0.00	0.00	0	0	7.00	-125	566	6.50	4260.70	4238.47
13.06.2009	11:53	23.88	200	110	37.80	37.80	210	120.96	6.10	45.40				
14.06.2009	12:50	48.83	195	115	37.90	37.90	3	124.10	6.73	-45				
15.06.2009	09:57	69.95	190	120	29.10	29.10	0	124.10	6.90	-47.60				
16.06.2009	09:58	93.96	185	125	36.20	36.20	0	134.43	6.86	-34.10				
17.06.2009	09:46	117.76	180	130	36.40	36.40	0	136.50	6.78	-116.40				
18.06.2009	09:51	141.84	175	135	46.60	46.60	3	153.49	6.84	-101				
19.06.2009	09:34	165.55	170	140	40.00	40.00	0	153.49	7.05	-85.60	1105		196.10	85.86

Table A. 88 Raw data for fluorescent light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	12:00	0.00	200	110	0.00	0.00	0	0	7.00	-125	566	6.50	4260.70	4238.47
13.06.2009	11:56	23.93	200	110	35.50	35.50	150	92.30	5.74	-42				
14.06.2009	12:53	48.88	192	118	41.00	41.00	5	103.68	6.87	-149.50				
15.06.2009	10:00	69.99	187	123	20.00	20.00	0	103.68	6.89	-102.70				
16.06.2009	09:59	93.97	182	128	15.00	15.00	0	103.68	6.89	-115.70				
17.06.2009	09:47	117.77	178	132	9.63	9.63	0	103.68	6.85	-152.80				
18.06.2009	09:53	141.87	173	137	15.10	15.10	0	111.65	6.91	-157.30				
19.06.2009	09:53	165.87	168	142	10.00	10.00	0	111.65	6.90	-119	1169		214.46	81.37

Table A. 89 Raw data for infrared light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	12:00	0.00	200	110	0.00	0.00	0	0	7.00	-125	566	6.50	4260.70	4238.47
13.06.2009	12:00	24.00	200	110	39.70	39.70	190	119.10	5.96	-32.70				
14.06.2009	12:55	48.92	195	115	41.00	41.00	22	131.60	6.64	-84.80				
15.06.2009	10:01	69.98	190	120	32.40	32.40	0	131.60	6.77	-114.10				
16.06.2009	10:03	94.01	185	125	25.80	25.80	0	131.60	6.75	-133.10				
17.06.2009	09:51	117.81	180	130	23.40	23.40	0	131.60	6.73	-143				
18.06.2009	09:57	141.91	175	135	23.00	23.00	0	131.60	6.74	-101.90				
19.06.2009	09:39	165.61	170	140	19.80	19.80	0	131.60	6.70	-89.50	1247		212.42	80.62

Table A. 90 Raw data for halogen light source

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	12:00	0.00	200	110	0.00	0.00	0	0	7.00	-125	0.56	6.50	4260.70	4238.47
13.06.2009	12:05	24.08	200	110	38.70	38.70	20	50.31	5.85	-32	1.74			
14.06.2009	12:57	48.95	193	117	48.20	48.20	5	66.54	6.73	-144.80	1.29	1.70		129.73
15.06.2009	10:04	70.06	188	122	54.80	54.80	8.30	81.55	6.72	-113.10				
16.06.2009	10:08	94.12	183	127	65.70	65.70	25.70	115.02	6.83	-114.80	1.11	0.90		75
17.06.2009	09:56	117.92	178	132	68.50	68.50	43	151.45	6.77	-145.30				
18.06.2009	09:48	141.78	173	137	70.00	70.00	30	177.93	6.81	-143.40	1.02	0.70		
19.06.2009	09:31	165.49	168	142	68.00	68.00	0.3	178.14	6.84	-163.20	1.14	0.70	168.75	76.10

Table A. 91 Raw data for sun light

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
12.06.2009	12:00	0.00	200	110	0.00	0.00	0	0	7.00	-125	566	6.50	4260.70	4238.47
13.06.2009	11:40	23.66	200	110	40.00	40.00	163	109.20	5.92	-204				
14.06.2009	13:02	49.02	179	131	45.00	45.00	2	125.05	6.78	-165.80				
15.06.2009	10:15	70.23	174	136	36.20	36.20	0	125.05	6.70	-113.20				
16.06.2009	10:13	94.19	169	141	36.00	36.00	0	125.05	6.77	-199				
17.06.2009	10:05	118.05	165	145	33.50	33.50	0	125.05	6.81	-174.50				
18.06.2009	10:02	142.00	160	150	21.40	21.40	0	125.05	6.77	-162.20				
19.06.2009	09:45	165.72	155	155	14.40	14.40	0	125.05	6.86	-139.50	1404		176.92	65.65

Table A. 92 Raw data for 1 klux halogen lamp

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	12:30	0.00	200	110	0.00	0.00	0	0	7.00	-160	264	7.66	4142	3996
02.07.2009	09:23	20.88	200	110	9.40	9.40	40	14.10	5.91	-162.60				
03.07.2009	09:18	44.79	195.6	114.4	24.80	24.80	22	37.58	6.50	-230.60				
04.07.2009	10:07	69.61	191.6	118.4	31.00	31.00	23	53.05	6.06	-255				
06.07.2009	09:19	116.81	186.6	123.4	33.20	33.20	3	58.31	6.60	-377.60				
07.07.2009	10:22	141.86	182.6	127.4	23.30	23.30	0	58.31	6.65	-335.34				
08.07.2009	09:11	165.09	181.6	128.4	12.40	12.40	0	58.31	6.60	-263.50				
09.07.2009	09:41	189.59	177.6	132.4	8.19	8.19	0	58.31	6.40	-437.70	2245		184	43

Table A. 93 Raw data for 3 klux halogen lamp

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	12:30	0.00	200	110	0.00	0.00	0	0	7.00	-160	264	7.66	4142	3996
02.07.2009	09:25	20.91	200	110	16.70	16.70	52	27.05	5.96	-144				
03.07.2009	09:20	44.83	194.5	115.5	31.30	31.30	38	56.72	6.70	-215.20				
04.07.2009	10:09	69.64	190.5	119.5	37.00	37.00	20	72.19	6.35	-265				
06.07.2009	09:21	116.84	181.5	128.5	26.20	26.20	0	72.19	6.80	-400				
07.07.2009	10:23	141.87	177.5	132.5	34.00	34.00	0	83.57	6.83	-363.70				
08.07.2009	09:12	165.05	176.5	133.5	32.60	32.60	0	83.57	6.77	-345.20				
09.07.2009	09:42	189.85	172.5	137.5	25.60	25.60	0	83.57	6.70	-470	2041		171	43.90

Table A. 94 Raw data for 5 klux halogen lamp

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	12:30	0.00	200	110	0.00	0.00	0	0	7.00	-160	264	7.66	4142	3996
02.07.2009	09:27	20.95	200	110	20.00	20.00	80	38	5.86	-171				
03.07.2009	09:22	44.87	195.5	114.5	38.20	38.20	42	75.78	6.45	-217				
04.07.2009	10:11	69.68	191.5	118.5	42.20	42.20	21	90.91	6.23	-225				
06.07.2009	09:23	116.83	186.5	123.5	20.00	20.00	0	90.91	6.56	-348				
07.07.2009	10:25	141.91	182.5	127.5	13.80	13.80	0	90.91	6.66	-316				
08.07.2009	09:13	164.71	181.5	128.5	4.92	4.92	0	90.91	6.60	-279				
09.07.2009	09:43	189.21	177.5	132.5	5.00	5.00	0	90.91	6.49	-442	1804		204	46

Table A. 95 Raw data for 7 klux halogen lamp

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	12:30	0.00	200	110	0.00	0.00	0	0	7.00	-160	264	7.66	4142	3996
02.07.2009	09:29	20.98	200	110	22.10	22.10	97	45.74	5.83	-228				
03.07.2009	09:23	44.88	194.5	115.5	37.80	37.80	39	79.83	6.50	-225				
04.07.2009	10:13	69.71	190.5	119.5	40.00	40.00	20	91.97	6.35	-233				
06.07.2009	09:25	116.91	181.5	128.5	18.40	18.40	0	91.97	6.86	-375				
07.07.2009	10:27	141.95	177.5	132.5	15.30	15.30	0	91.97	6.83	-335				
08.07.2009	09:15	165.15	176.5	133.5	6.81	6.81	0	91.97	6.79	-322				
09.07.2009	09:44	189.63	172.5	137.5	6.33	6.33	0	91.97	6.82	-445	2090		169	48

Table A. 96 Raw data for 10 klux halogen lamp

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2, i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	12:30	0.00	200	110	0.00	0.00	0	0	7	-160	264	7.66	4142	3996
02.07.2009	09:34	21.06	200	110	26.60	26.60	160	71.82	5.63	-165	1415			403.15
03.07.2009	09:26	44.93	195.6	114.4	41.90	41.90	40	107.25	6.51	-223	1692	1.83		76.25
04.07.2009	10:14	69.73	190.6	119.4	39.60	39.60	10	111.21	6.40	-235				
06.07.2009	09:26	116.93	181.6	128.4	13.30	13.30	0	111.21	7	-370.10	1543	1.83		71.51
07.07.2009	10:28	141.96	173.6	136.4	3.50	3.50	0	111.21	7	-280				
08.07.2009	09:16	165.16	172.6	137.4	3.27	3.27	0	111.21	6.95	-281.10				
09.07.2009	09:46	189.66	168.6	141.4	2.88	2.88	0	111.21	6.93	-443.50	1701	1	163.38	59.42

Table A. 97 Raw data for sunlight

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
01.07.2009	12:30	0.00	200	110	0.00	0.00	0	0	7.00	-160	264	7.66	4142	3996
02.07.2009	09:17	20.78	200	110	12.40	12.40	40	18.60	5.96	-206				
03.07.2009	09:06	44.60	196.6	113.4	26.00	26.00	23	40.42	6.50	-299				
04.07.2009	09:57	69.45	192.6	117.4	35.70	35.70	20.50	60.17	6.09	-144				
06.07.2009	09:34	117.06	186.6	123.4	32.70	32.70	0	60.17	7.00	-395				
07.07.2009	10:19	141.81	182.6	127.4	28.00	28.00	0	60.17	7.00	-396				
08.07.2009	09:42	165.20	181.6	128.4	21.60	21.60	0	60.17	7.02	-373				
09.07.2009	10:03	189.55	177.6	132.4	18.50	18.50	0	60.17	6.76	-438	1744		168	47

Table A. 98 Raw data for 30min light/30dark lighting regime

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
23.07.2009	13:40	0.00	200	110	0.00	0.00	0	0	7.10	-175	232		5951	3731
24.07.2009	09:23	19.72	200	110	35.50	35.50	182	103.66	5.50	-151.70				
25.07.2009	07:21	41.79	193.2	116.8	34.70	34.70	0	103.66	7.10	-185.20				
27.07.2009	09:27	91.89	186.4	123.6	27.90	27.90	0	103.66	7.23	-204.20				
28.07.2009	09:58	116.41	181.4	128.6	30.00	30.00	0	107.75	7.40	-114.90				
29.07.2009	09:32	139.81	180.4	129.6	31.60	31.60	0	110.12	7.30	-131.80				
30.07.2009	09:43	163.99	179.4	130.6	26.00	26.00	0	110.12	7.14	-323.80	3371		249.45	66.69

Table A. 99 Raw data for 2h light/2h dark lighting regime

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
23.07.2009	13:40	0.00	200	110	0.00	0.00	0	0	7.10	-175	232		5951	3731
24.07.2009	09:25	19.75	200	110	38.40	38.40	200	119.04	5.33	-126.60				
25.07.2009	07:22	41.69	191.3	118.7	51.10	51.10	43	159.42	6.50	-210.20				
27.07.2009	09:28	91.79	185.3	124.7	37.00	37.00	0	159.42	6.75	-271.60				
28.07.2009	10:00	116.32	180.3	129.7	15.00	15.00	0	159.42	6.75	-134.90				
29.07.2009	09:34	139.88	179.3	130.7	10.00	10.00	0	159.42	6.80	-107.50				
30.07.2009	09:46	164.08	178.3	131.7	6.20	6.20	0	159.42	6.71	-207	3180		161.33	59.77

Table A. 100 Raw data for 4h light/4h dark lighting regime

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
23.07.2009	13:40	0.00	200	110	0.00	0.00	0	0	7.10	-175	232		5951	3731
24.07.2009	09:27	19.79	200	110	36.40	36.40	200	112.84	5.23	-361.20				
25.07.2009	07:25	41.82	194.8	115.2	56.60	56.60	50	166.30	6.47	-289				
27.07.2009	09:30	91.90	188.8	121.2	31.60	31.60	0	166.30	6.88	-322.60				
28.07.2009	10:02	116.43	183.8	126.2	14.30	14.30	0	166.30	7.02	-204.70				
29.07.2009	09:36	139.99	182.8	127.2	8.70	8.70	0	166.30	7.00	-144.40				
30.07.2009	09:49	164.20	181.8	128.2	1.00	1.00	0	166.30	6.97	-227	3000		181.14	6.90

Table A. 101 Raw data for 6h light/6h dark lighting regime

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
23.07.2009	13:40	0.00	200	110	0.00	0.00	0	0	7.10	-175	232		5951	3731
24.07.2009	09:29	19.82	200	110	38.30	38.30	168	106.47	5.32	-386.90				
25.07.2009	07:26	41.75	193.8	116.2	53.60	53.60	102	181.29	6.37	-257				
27.07.2009	09:32	91.85	186.8	123.2	28.50	28.50	0	181.29	6.96	-334.60				
28.07.2009	10:03	116.36	181.8	128.2	16.80	16.80	0	181.29	7.10	-151.40				
29.07.2009	09:37	139.92	180.8	129.2	5.95	5.95	0	181.29	7.08	-123.20				
30.07.2009	09:50	164.14	179.8	130.2	5.94	5.94	0	181.29	7.12	-164	2697		133.12	72.71

Table A. 102 Raw data for 12h light/12h dark lighting regime

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
23.07.2009	13:40	0.00	200	110	0.00	0.00	0	0	7.10	-175	232		5951	3731
24.07.2009	09:31	19.85	200	110	36.90	36.90	160	99.63	5.40	-380				
25.07.2009	07:28	41.81	193	117	51.10	51.10	10	123.94	6.60	-314				
27.07.2009	09:35	91.92	187.3	122.7	53.90	53.90	0.20	130.39	7.00	-232				
28.07.2009	10:04	116.40	182.3	127.7	35.00	35.00	0	130.39	7.20	-184				
29.07.2009	09:39	139.38	181.3	128.7	24.40	24.40	0	130.39	7.10	-214				
30.07.2009	09:51	163.58	179.3	130.7	15.00	15.00	0	130.39	7.10	-275	3047		255	67.83

Table A. 103 Raw data for continuous lighting regime

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2, i(%)	CH_2 , i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Starch (mgL^{-1})	Glucose (mgL^{-1})
23.07.2009	13:40	0.00	200	110	0.00	0.00	0	0	7.10	-175	232		5951	3731
24.07.2009	09:35	19.72	200	110	37.70	37.70	200	116.87	5.41	-366.50				
25.07.2009	07:30	41.79	195	115	59.50	59.50	102	204.51	6.74	-258.10				
27.07.2009	09:36	91.89	188	122	61.20	61.20	0.60	211.12	6.81	-285.40				
28.07.2009	10:06	116.41	183	127	43.00	43.00	0	211.12	7.07	-168.50				
29.07.2009	09:40	139.81	182	128	33.40	33.40	0	211.12	7.08	-206.90				
30.07.2009	09:53	163.99	181	129	31.80	31.80	0	211.12	7.10	-297.80	2578		147.47	77.58

A.2 Raw Data for Continuous Experiments

A.2.1 Raw Data for the Variable Hydraulic Residence Time Experiments

Table A. 104 Raw data for HRT=6 days obtained from the inner cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
06.10.2009	17:00	0	0	2047.45	0.00	0.00	0	0	7.08	-320	85	0,289	1136	5000
07.10.2009	11:22	18.36	1522.5	524.93	4.50	4.50	80	27.22	6.40	-194		0.60	64.30	370
07.10.2009	12:38	0	0	2047.45	0.00	0.00	0	0	7.30	-215				
08.10.2009	11:33	22.91	1582.6	464.83	7.78	7.78	60	40.83	7.10	-130.80	2799	0.40	49.50	235
08.10.2009	12:50	0	126.02	1921.43	0.32	0.32	0	0	7.10	-130.80				
09.10.2009	11:37	22.78	961.07	1086.38	4.94	4.94	265	60.60	6.40	-130	2235	0.30	57.20	187
09.10.2009	12:50	0	126.02	1921.43	0.32	0.32	0	0	7.20	-139				
10.10.2009	11:37	22.78	961.07	1086.38	4.94	4.94	265	60.60	6.90	-147	2735	0.70	48.90	265
10.10.2009	11:58	0	1592.9	454.53	7.08	7.08	0	0	6.88	-82				
11.10.2009	12:27	24.48	1592.9	454.53	11.68	11.68	80	30.25	7.08	-82	2264	0	42.20	124
11.10.2009	12:27	0	1592.9	454.53	11.68	11.68	0	0	7.08	-121				
12.10.2009	11:45	23.3	1592.9	454.53	14.20	14.20	20	14.29	6.40	-124	2652	0.80	51.20	149

Table A. 105 Raw data for HRT=6 days obtained from the outer cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
06.10.2009	17:00	0	2554.2	3030.68	0.00	0.00	0	0	7.08	-320	85	0,28	1136	5000
07.10.2009	11:19	18.32	1031.7	4553.2	2.10	2.10	900	114.51	6.40	-194		0.60	64.30	370
07.10.2009	12:40	0	2554.2	3030.68	0.26	0.26	0	0	7.30	-215				
08.10.2009	11:35	22.91	971.6	4613.3	3.68	3.68	100	165.56	7.10	-130.80	2799	0.40	49.50	235
08.10.2009	12:51	0	2428	3156.92	0.27	0.27	0	0	7.10	-130.80				
09.10.2009	11:38	22.78	1592.9	3991.98	2.88	2.88	265	114.07	6.40	-130	2235	0.30	57.20	187
09.10.2009	12:51	0	2428	3156.92	0.27	0.27	0	0	7.20	-139				
10.10.2009	11:38	22.78	1592.9	3991.98	2.88	2.88	265	114.07	6.90	-147	2735	0.70	48.90	265
10.10.2009	12:02	0	961.07	4623.83	6.31	6.31	0	0	6.88	-82				
11.10.2009	12:30	24.46	961.07	4623.83	8.58	8.58	121	115.34	7.08	-82	2264	0	42.20	124
11.10.2009	12:30	0	961.07	4623.83	8.58	8.58	0	0	7.08	-121				
12.10.2009	11:47	23.28	961.07	4623.83	12.00	12.00	45	163.53	6.40	-124	2652	0.80	51.2	149

Table A. 106 Raw data for HRT=5 days obtained from the inner cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
20.10.2009	12:40	0	884.76	1162.69	0.35	0.35	0	0	7.30	-310	3950	0.45	38	149
21.10.2009	11:58	23.3	1613.15	434.3	4.68	4.68	310	30.76	7.10	-162	3333	0.30	36	182
21.10.2009	12:50	0	126.02	1921.43	0.32	0.32	0	0	7.20	-174				
22.10.2009	11:37	22.78	961.07	1086.38	4.94	4.94	265	60.60	7.18	-150	2200	0.35	31	156
22.10.2009	12:25	0	178.95	1868.5	0.26	0.26	0	0	7.20	-162				
23.10.2009	13:26	25.01	1643.5	403.95	6.93	6.93	150	33.53	7.00	-180	2338	0.90	30	197
23.10.2009	12:01	0	196.54	1850.91	0.28	0.28	0	0	7.30	-185				
24.10.2009	14:23	26.38	1542.34	505.11	7.11	7.11	90	37.12	6.86	-210	2373	1.10	20	135
24.10.2009	14:54	0	85.35	1962.1	0.38	0.38	0	0	7.30	-191				
25.10.2009	13:24	23.85	1542.34	505.11	7.08	7.08	90	34.67	7.00	-161	2661	0.25	21	146
25.10.2009	13:48	0	105.79	1941.66	0.37	0.37		0	7.30	-183.20				
26.10.2009	13:48	0	1542.34	505.11	6.76	6.76	80	32.36	7.10	-81.80	2814	0.60	19	137

Table A. 107 Raw data for HRT=5 days obtained from the outer cylinder

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH_2. i(%)**	**CH_2. i-1(%)**	**Vw (mL)**	**VH_2 (mL)**	**pH**	**ORP (mV)**	**TVFA (mgL^{-1})**	**NH_4-N (mgL^{-1})**	**Glucose (mgL^{-1})**	**Starch (mgL^{-1})**
20.10.2009	12:37	0	1669.24	3915.66	0.50	0.50	0	0	7.30	-310	3950	0.45	38	149
21.10.2009	12:02	23.42	940.84	4644.06	2.82	2.82	310	120.12	7.10	-162	3333	0.30	36	182
21.10.2009	12:51	0	2427.98	3156.92	0.27	0.27	0	0	7.20	-174				
22.10.2009	11:38	22.78	1592.92	3991.98	2.88	2.88	265	114.07	7.18	-150	2200	0.35	31	156
22.10.2009	12:26	0	2375.05	3209.85	0.27	0.27	0	0	7.20	-162				
23.10.2009	13:28	25.03	910.49	4674.41	3.68	3.68	150	168.87	7.00	-180	2338	0.90	30	197
23.10.2009	12:02	0	2357.16	3227.74	0.31	0.31	0	0	7.30	-185				
24.10.2009	14:23	26.34	1011.66	4573.24	3.38	3.38	90	147.61	6.86	-210	2373	1.10	20	135
24.10.2009	14:55	0	2468.45	3116.45	0.33	0.33	0	0	7.30	-191				
25.10.2009	13:26	22.47	1011.66	4573.24	3.09	3.09	90	133.80	7.00	-161	2661	0.25	21	146
25.10.2009	13:49	0	2448.21	3136.69	1.09	1.09		0	7.30	-183.20				
26.10.2009	13:41	23.86	1011.66	4573.24	3.34	3.34	80	121.22	7.10	-81.80	2814	0.60	19	137

Table A. 108 Raw data for HRT=4 days obtained from the inner cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
30.10.2009	14:28	0	105.78	1941.67	0.37	0.37	0	0	7.30	-106	2814	0.60	19	137
31.10.2009	14:26	23.98	1532.23	515.22	6.60	6.60	190	39.36	6.98	-191	3246	0.15	23	177
01.11.2009	13:21	0	136.14	1911.31	0.00	0.00	0	0	7.40	-172				
02.11.2009	11:51	22.5	1542.34	505.11	7.00	7.00	80	40.95	7.17	-146.60	2637	0.15	24	
02.11.2009	12:26	0	75.44	1972.01	0.00	0.00	0	0	7.30	-186.50				
03.11.1900	12:40	24.23	1552.46	494.99	6.87	6.87	105	41.21	7.00	-158.50	2403	0.10	26	
03.11.2009	13:32	0	115.9	1931.55	0.00	0.00	0	0	7.40	-200				
04.11.2009	12:39	23.11	1542.32	505.13	7.00	7.00	120	43.75	7.15	2	3405	0.10	25	183
04.11.2009	13:15	0	105.79	1941.66	0.00	0.00	0	0	7.30	-150				
05.11.2009	14:13	24.96	1552.6	494.85	8.57	8.57	40	45.83	7.20	-210	3873	0.15	24	
05.11.2009	15:10	0	75.44	1972.01	0.00	0.00	0	0	7.50	-247.50				
06.11.2009	13:14	22.07	1582.81	464.64	7.17	7.17	120	41.91	7.00	-185				

Table A. 109 Raw data for HRT=4 days obtained from the outer cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
30.10.2009	14:29	0	2448.21	3136.69	0.27	0.27	0	0	7.30	-106	2814	0.60	19	137
31.10.2009	14:28	23.98	1021.77	4563.13	3.00	3.00	180	133.82	6.98	-191	3246	0.15	23	177
01.11.2009	13:22	0	2417.86	3167.04	0.31	0.31	0	0	7.40	-172				
02.11.2009	11:52	22.5	1011.66	4573.24	3.10	3.10	120	135.67	7.17	-146.60	2637	0.15	24	
02.11.2009	12:27	0	2478.56	3106.34	0.27	0.27	0	0	7.30	-186.50				
03.11.1900	12:41	23.76	1001.54	4583.36	2.95	2.95	140	130.95	7.00	-158.50	2403	0.10	26	
03.11.2009	13:33	0	2438	3146.9	0.00	0.00	0	0	7.40	-200				
04.11.2009	12:40	24.11	1011.66	4573.24	2.95	2.95	140	139.04	7.15	2	3405	0.10	25	183
04.11.2009	13:16	0	2448.21	3136.69	0.26	0.26	0	0	7.30	-150				
05.11.2009	14:16	25	1001.54	4583.36	3.57	3.57	77	158.21	7.20	-210	3873	0.15	24	
05.11.2009	15:11	0	2478.46	3106.44	0.00	0.00	0	0	7.50	-247.50				
06.11.2009	13:15	22.06	971.16	4613.74	2.97	2.97	160	141.78	7.00	-185				

Table A. 110 Raw data for HRT=3 days obtained from the inner cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
09.11.2009	16:45	0	257.54	1789.91	0.38	0.38	0	0	7.45	-170	3047	0.3	25	
10.11.2009	14:36	21.85	1613.2	434.29	8.57	8.57	125	41.12	7.09	-50				
10.11.2009	15:12	0	257.54	1789.91	0.35	0.35	0	0	7.45	-190				
11.11.2009	14:51	23.65	1572.7	474.76	9.19	9.19	210	56.66	7.15	-230				
11.11.2009	15:37	0	227.18	1820.27	0.37	0.37	0	0	7.50	-235				
12.11.2009	12:46	21.14	1592.9	454.53	8.85	8.85	120	44.11	7.00	-230	2367	0.33	21	286
12.11.2009	13:28	0	247.42	1800.03	0.37	0.37	0	0	7.50	-270				
13.11.2009	13:18	23.86	1592.9	454.53	9.68	9.68	160	52.82	7.10	-219				
13.11.2009	11:57	0	247.42	1800.03	0.21	0.21	0	0	7.50	-250				
14.11.2009	12:26	24.48	1592.9	454.53	6.00	6.00	80	29.92	6.90	-174				
14.11.2009	12:45	0	126.02	1921.43	0.32	0.32	0	0	7.50	-243				
15.11.2009	11:32	22.78	961.07	1086.38	4.94	4.94	265	60.60	6.80	-163				

Table A. 111 Raw data for HRT=3 days obtained from the outer cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
09.11.2009	16:46	0	2296.5	3288.44	0.43	0.43	0	0	7.45	-170	3047	0.30	25	
10.11.2009	14:38	21.86	940.84	4644.06	3.30	3.30	140	143.73	7.09	-50				
10.11.2009	15:14	0	2296.5	3288.44	0.27	0.27	0	0	7.45	-190				
11.11.2009	14:53	23.75	981.31	4603.59	3.87	3.87	165	175.66	7.15	-230				
11.11.2009	15:38	0	2326.8	3258.09	0.28	0.28	0	0	7.50	-235				
12.11.2009	12:49	21.18	961.08	4623.82	3.65	3.65	160	165.48	7.00	-230	2367	0.33	21	286
12.11.2009	13:29	0	2306.6	3278.32	0.29	0.29	0	0	7.50	-270				
13.11.2009	13:21	23.86	961.08	4623.82	4.11	4.11	160	187.10	7.10	-219				
13.11.2009	11:58	0	2306.6	3278.32	0.26	0.26	0	0	7.30	-215				
14.11.2009	12:28	24.50	961.08	4623.82	3.68	3.68	100	165.31	7.10	-130.80				
14.11.2009	12:47	0.00	2428	3156.92	0.33	0.33	0	0	7.30	-191				
15.11.2009	11:35	22.97	1592.9	3991.97	3.50	3.50	120	133.50	7.00	-161				

Table A. 112 Raw data for HRT=2 days obtained from the inner cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
16.11.2009	13:59	0	227.19	1820.26	0.43	0.43	0	0	7.40	-175.00				
17.11.2009	12:44	22.75	1643.51	403.94	9.00	9.00	160	42.92	6.93	-200.00				
17.11.2009	13:33	0	186.72	1860.73	0.36	0.36	0	0	7.40	-218.00				
18.11.2009	15:00	24.45	1643.51	403.94	10.00	10.00	400	73.69	6.80	-100.00				
18.11.2009	15:39	0	308.12	1739.33	0.38	0.38	0	0	7.40	-170.00				
19.11.2009	11:56	20.28	1643.51	403.94	10.00	10.00	170	50.80	6.98	-173.00				
19.11.2009	12:38	0	257.54	1789.91	0.37	0.37	0	0	7.35	-190.50				
20.11.2009	11:12	22.56	1643.51	403.94	10.00	10.00	320	65.77	7.05	-219.00				
20.11.2009	11:41	0	389.05	1658.4	0.38	0.38	0	0	7.50	-244.50				
21.11.2009	13:12	25.51	1683.96	363.49	10.00	10.00	35	33.54	6.90	-236.50				
21.11.2009	14:00	0	298	1749.45	0.26	0.26	0	0	7.40	-317.00				
22.11.2009	12:23	22.38	1694.08	353.37	9.36	9.36	362	62.41	7.05	-162.00	2817.00	0.30	28.80	325.69

Table A. 113 Raw data for HRT=2 days obtained from the outer cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
16.11.2009	14:00	0	2326.81	3258.09	0.33	0.33	0	0	7.40	-175				
17.11.2009	12:48	22.6	910.49	4674.41	3.87	3.87	162	176.41	6.93	-200				
17.11.2009	13:35	0	2367.28	3217.62	0.64	0.64	0	0	7.40	-218				
18.11.2009	15:02	25.44	910.49	4674.41	5.38	5.38	160	239.49	6.80	-100				
18.11.2009	15:40	0	2245.88	3339.02	0.61	0.61	0	0	7.40	-170				
19.11.2009	11:59	20.32	910.49	4674.41	4.53	4.53	160	198.63	6.98	-170				
19.11.2009	12:39	0	2296.46	3288.44	0.40	0.40	0	0	7.35	-190.50				
20.11.2009	11:14	22.58	910.49	4674.41	5.30	5.30	160	243.06	7.05	-219				
20.11.2009	11:42	0	2164.95	3419.95	0.59	0.59	0	0	7.50	-244.50				
21.11.2009	13:09	25.45	870.02	4714.88	5.11	5.11	460	244.25	6.90	-236.50				
21.11.2009	14:01	0	2256	3328.9	0.30	0.30	0	0	7.40	-317				
22.11.2009	12:23	22.37	859.91	4724.99	4.74	4.74	140	220.61	7.05	-162	2817	0.30	28.80	325.69

Table A. 114 Raw data for HRT=1 days obtained from the inner cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
22.11.2009	13:35	0	399.17	1648.28	0.38	0.38	0	0	7.40	-215.10				
23.11.2009	11:21	21.77	1714.33	333.12	9.61	9.61	520	75.72	6.76	-219				
23.11.2009	12:39	0	480.10	1567.35	0.28	0.28	0	0	7.40	-219.10				
24.11.2009	10:53	22.23	1795.25	252.20	10.00	10.00	435	64.33	6.76	-162				
24.11.2009	11:46	0	449.75	1597.70	0.39	0.39	0	0	7.50	-246				
25.11.2009	10:33	22.79	1795.25	252.20	10.00	10.00	560	74.98	6.80	-161	2850	0.20	31.52	445
25.11.2009	11:40	0	510.45	1537	0.40	0.40	0	0	7.50	-233.40				
26.11.2009	09:15	21.59	1845.84	201.61	10.00	10.00	620	76.01	6.70	-162.50				

Table A. 115 Raw data for HRT=1 days obtained from the outer cylinder

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH_2. i(%)	CH_2. i-1(%)	Vw (mL)	VH_2 (mL)	pH	ORP (mV)	TVFA (mgL^{-1})	NH_4-N (mgL^{-1})	Glucose (mgL^{-1})	Starch (mgL^{-1})
22.11.2009	13:36	0	2154.83	3430.07	0.29	0.29	0	0	7.40	-215.10				
23.11.2009	11:24	21.8	839.67	4745.23	5.49	5.49	145	258.52	6.76	-219.00				
23.11.2009	12:40	0	2073.903	3510.997	0.38	0.38	0	0	7.40	-219.10				
24.11.2009	10:55	22.25	758.745	4826.155	5.83	5.83	160	276.63	6.76	-162.00				
24.11.2009	11:48	0	2104.25	3480.65	0.51	0.51	0	0	7.50	-246.00				
25.11.2009	10:35	22.78	758.74	4826.16	6.46	6.46	155	304.03	6.80	-161.00	2850	0.20	31.52	445
25.11.2009	11:40	0	2043.55	3541.35	0.33	0.33	0	0	7.50	-233.40				
26.11.2009	09:18	21.61	708.16	4876.74	6.79	6.79	160	330.30	6.70	-162.50				

B.1 Nomenclature

A	Axial point in Box-Wilson statistical experiment design
b	Coefficient of response equation
$C_{H2,i}$	H_2 content in the head space of the ith measurement, %
$C_{H2,i-1}$	H_2 content in the head space of the $i\text{-}1^{th}$ measurement, %
CHF	Cumulative hydrogen formation, mL
F	Factorial point in Box-Wilson statistical experiment design
ΔGo	Gibbs free energy, kj
H	Cumulative hydrogen at any time, mL
ΔH	Produced cumulative hydrogen, moles or mL
HPR	Hydrogen production rate, mLH_2h^{-1}
HRT	Hydraulic residence time, h
HY	Hydrogen yield, $molH_2$ $molglucose^{-1}$
I	Light intensity, lux
Im	Maximum light intensity, lux
K	Temperature, Kelvin
Ks	Saturation constant, gL^{-1}
K_I	Substrate inhibition constant, gL^{-1}
k	Specific hydrogen production rate constant, mLH_2g^{-1}biomass h^{-1}
ks	Light stimulation constant, mLH_2 lux^{-1} h^{-1} $biomass^{-1}$
ki	Light inhibition constant, mLH_2 lux^{-1} h^{-1} $biomass^{-1}$
λ	Lag phase, hour
μ_{max}	Maximum specific growth rate, h^{-1}
mV	Mili volt
n	Mole number, mol
ORP	Oxidation reduction potential, mV
P	Maximum hydrogen formation potential, mL
Q	Flowrate, liter d^{-1}
R	Hydrogen formation rate, mLH_2h^{-1}
Rm	Maximum hydrogen formation rate, mLH_2h^{-1}
Rx	Specific hydrogen formation rate, mLH_2 g^{-1} biomass h^{-1}
STP	Standard temperature and pressure, 0^oC and 1 atm unless otherwise specified
So	Initial starch concentration, gL^{-1}
Sf	Final starch concentration, gL^{-1}
SHPR	Specific hydrogen production rate, mLH_2 g^{-1} biomass h^{-1}

ΔS	Consumed substrate, mole glucose or g starch
t	Time, h
Vo	Initial volume of fermentation broth, liter
$V_{H2,i}$	Volume of cumulative H_2 calculated at i^{th} measurement, mL
$V_{H2,i-1}$	Volume of cumulative H_2 calculated at $i\text{-}1^{th}$ measurement, mL
V_w	Total gas volume measured by water displacement method, mL
V_{Gi}	Volume of gas in the head space of the bottle for the ith measurement, mL
V_{Gi-1}	Volume of gas in the head space of the bottle for the $i\text{-}1^{th}$ measurement, mL
V_l	Volume of fermentation medium, mL
V_g	Head space volume of fermentation reactor, mL
VHPR	Volumetric hydrogen production rate, $mLH_2\ l^{-1}\ d^{-1}$
w	Weight, g
v_{max}	Maximum hydrogen production rate, $mLH_2\ l^{-1}\ h^{-1}$
X_o	Initial biomass concentration, gL^{-1}
X_D	Initial biomass concentration of dark fermentation bacteria, gL^{-1}
X_L	Initial biomass concentration of photo fermentative bacteria, gL^{-1}
X_D/X_L	Dark to light biomass concentration ratio
X_T	Total initial biomass concentration, gL^{-1}
X	Independent variable in Box-Wison statistical experiment design
Y	Objective function in Box-Wison statistical experiment design

Abbreviations

ANS	Anaerobic sludge
ATP	Adenosin triphosphate
ave	average
C/N	Carbon to nitrogen ratio
C/P	Carbon to phosphorous ratio
CSTR	Completely stirred reactor
CAB	*Clostridium acetobutylicum*
CB	*Clostridium butyricum*
DFE	Dark fermentation effluent
DOE	US Department of Energy
DSMZ	Deutsche Sammlung von Microorganismen und Zellkulturen
dw	Dry weight

EA	*Enterobacter aerogenes*
EDTA	Ethylenediaminetetraacetic acid
EFES	Anaerobic granular sludge of EFES beer industry company
GC	Gas choromatography
HPLC	High performance liquid chromatography
HAc	Acetic acid
HBu	Butyric acid
HPr	Propionic acid
HLa	Lactic acid
IR	Infrared lamp
MIX	Mixture of Pak Maya and EFES anaerobic sludges
NRLL	US National Centre for Agricultural Utilization Research
OD	Optical density
PAK	Anaerobic sludge of Pak Maya Baekers Yeast Company
PHB	Polyhydroxybutyrate
PNS	Purpule non-sulphur bacteria
RS	*Rhodobacter sphaeroides*
SBR	Sequencing batch reactor
STR	Starch
TVFA	Total volatile fatty acid
TSG	Total sugar
Tung	Tungsten lamp
UV	Ultraviolet
VFA	Volatile fatty acid
ww	wastewater
WP	Wheat powder
WPS	Wheat powder solution