DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOHYDROGEN PRODUCTION BY DARK / LIGHT FERMENTATION OF HYDROLYSED WHEAT STARCH

by
Rana SAĞNAK

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İZMİR

BIOHYDROGEN PRODUCTION BY DARK / LIGHT FERMENTATION OF HYDROLYSED WHEAT STARCH

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We have read the thesis entitled **"BIOHYDROGEN PRODUCTION BY DARK / LIGHT FERMENTATION OF HYDROLYSED WHEAT STARCH"** completed by **RANA SAĞNAK** 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 Master of Science.

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ABSTRACT

Biological hydrogen gas production from acid hydrolyzed wheat starch (AHWS) solution was investigated using batch dark and continuous dark, photo and combined fermentation systems. Hydrogen production yield and specific hydrogen production rate were considered as the criteria for performance comparison.

In batch dark fermentation experiments, initial waste wheat and biomass concentrations on hydrogen gas production rate and yield were investigated using heat pre-treated anaerobic sludge (ANS) and acid hydrolyzed wheat starch. Continuous experiments of dark fermentation were performed to investigate the effects of hydraulic residence time (HRT) on hydrogen gas production rate and yield.

Hydrogen gas production by light fermentation using volatile fatty acid (VFA) containing batch dark fermentation effluent (DFE) was investigated. The effects of hydraulic residence time (HRT) on hydrogen gas production rate and yield were investigated using pure culture of *Rhodobacter sphaeroides* NRLL-1727.

In combined dark and photo fermentations of AHWS, the effects of hydraulic residence time (HRT) on hydrogen gas production rate and yield were investigated by using heat pre-treated anaerobic sludge (ANS) and pure culture of *Rhodobacter sphaeroides* NRLL-1727. Continuous experiments were performed by periodic feeding and effluent removal.

Keywords: Bio-hydrogen, acid hydrolysed wheat starch, hydraulic residence time, batch and continuous operation, dark fermentation, photo fermentation, combined fermentation.

HİDROLİZE EDİLMİŞ BUĞDAYDAN IŞIKLI IŞIKSIZ FERMENTASYONLA HİDROJEN GAZ ÜRETİMİ

ÖZ

Bu çalışmada öğütülmüş atık buğdaydan kesikli ve sürekli işletilen ışıksız, ışıklı ve birleşik fermentasyonla hidrojen gazı üretimi araştırılmıştır. Kesikli deneyler ışıksız fermentasyon için yapılırken, sürekli deneyler ışıksız, ışıklı ve birleşik fermentasyon için yapılmıştır. Performans kıyaslama kriterleri olarak hidrojen üretim verimi ve özgül hidrojen üretim hızı seçilmiştir.

Kesikli ışıksız fermentasyonda, ısıl işleme tabi tutulmuş anaerobik çamur (ANS) ve asit ile hidrolize edilmiş buğday çözeltisi (AHWS) kullanılarak degişik başlangıç atık buğday ve biyokütle konsantrasyonlarında deneyler yapılmışdır. Sürekli ışıksız fermentasyonda ise hidrolik alıkonma süresinin hidrojen üretim hızı ve verimi üzerine etkileri incelenmiştir.

Uçucu yağ asitleri içeren kesikli ışıksız fermentasyon çıkış suyundan, sürekli ışıklı fermentasyon ile hidrojen üretimi deneyleri yapılmıştır. *Rhodobacter sphaeroides* NRLL-1727 saf kültürü kullanılarak, hidrolik alıkonma süresinin hidrojen üretim hızı ve verimi üzerine etkileri incelenmiştir.

Sürekli ışıksız ve ışıklı birleşik fermentasyonla hidrolize atık buğday nişastasından hidrojen üretim hızı ve verimi üzerine hidrolik alıkonma suresi etkileri incelenmişdir. Bu deneylerde ısıl işleme tabi tutulmuş anaerobik çamur (ANS) ve *Rhodobacter sphaeroides* NRLL-1727 saf kültürü kullanılmışdır.

Anahtar Kelimeler: Biyohidrojen, asit hidrolize edilmiş buğday nişastası, hidrolik alıkonma süresi, kesikli ve sürekli işletme, ışıksız (karanlık) fermentasyon, ışıklı fermentasyon, birleşik fermentasyon.

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CHAPTER ONE
INTRODUCTION

1.1 Bio-Hydrogen Production Methods

Due to significant global climate changes, considerable environmental problems, decreasing reserves of fossil fuels and increasing energy demand stimulated a search for renewable and non-polluting energy sources. It is widely believed that hydrogen is an attractive energy carrier of the future with a high energy content of 122 kJ g^{-1} which is about 2.75 times greater than fossil fuels (Das & Veziroglu, 2001; Han & Shin, 2004; Kapdan & Kargi, 2006; Kotay & Das, 2008; Zhang & Shen, 2005) . Hydrogen gas is a clean fuel only produces water vapor with no CO_x , SO_x and NO_x emissions when it combusted. However, unlike fossil fuels and natural gas, hydrogen gas is not readily available in nature and requires expensive production methods (Kapdan & Kargi, 2006). Various pathways such as steam reforming and thermal cracking of natural gas; coal gasification and non- catalytic partial oxidation of fossil fuels; electrolysis and photolysis of water and thermochemical cycles were used for hydrogen gas production (Das & Veziroglu, 2001; Kapdan & Kargi, 2006; Manish & Banerjee, 2008). Almost all hydrogen production methods are based on utilization of fossil fuels, which are associated with release of large quantities of greenhouse gases. Therefore, current hydrogen production processes need to be replaced with a renewable and environmentally harmless process.

Operating under mild temperature and pressure, biological processes for hydrogen gas production are not only environmentally friendly but also provide waste treatment and require less energy inputs. Kapdan & Kargi, (2006) summarized the waste materials to be used for fermentative hydrogen gas production which are mainly starch and cellulose containing agricultural or food industry wastes, carbohydrate rich industrial wastewaters and waste sludge from wastewater treatment plants.

Hydrogen can be produced biologically by biophotolysis (direct and indirect), photo fermentation, dark fermentation and by a combination of dark- and photo-fermentation (Das & Veziroglu, 2001; Kapdan & Kargi, 2006).

Biophotolysis is classified in two distinctive ways, direct and indirect biophotolysis. During direct biophotolysis, by using the light as an energy source, green algae split water into molecular H_2 and O_2. (Eqn.1.1). Since all biological hydrogen production processes use the enzyme hydrogenase and nitrogenase as hydrogen producing protein (Das & Veziroglu, 2001) oxygen generated as a byproduct is a powerful suppressor of all H_2-related reactions (Eroglu & Melis, 2011). Kapdan & Kargi (2006) reported that inhibition of the hydrogenase enzyme by oxygen can be alleviated by cultivation of algae under sulfur deprivation for 2–3 days to provide anaerobic conditions in the light. Das & Veziroglu, (2008) also reported that some green algae such as *Dunaliella salina* and *Chlorella vulgaris* do not have hydrogenase activity as compared to other green algae like *Scenedesmus obliquus, Chlorococcum littorale, Platymonas subcordiformis* and *Chlorella fusca.*

$$2\ H_2O + \text{light energy} \rightarrow 2\ H_2 + O_2 \qquad \text{Eqn.1.1}$$

Cyanobacteria (also known as blue-green algae, cyanophyceae, or cyanophytes) are a large and diverse group of photoautotrophic microorganisms and responsible for hydrogen production via indirect biophotolysis (Levin, Pitt, & Love, 2004). Using the light as an energy source, cyanobacteria fixes CO_2 from air to generate carbohydrates which are used to produce hydrogen as summarized in equations 1.2 and 1.3. Cyanobacterias are also capable of fixation of atmospheric nitrogen. Some well-known Cyanobacterias are *Anabaena species*, *Calothrix sp.*, *Oscillatoria sp.*, *Synechococcus sp*., *Gloebacter sp*. and *Chlamidomonas*.

$$6\ H_2O + 6\ CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2 \qquad \text{Eqn 1.2}$$

$$C_6H_{12}O_6 + 6\ H_2O + \text{light energy} \rightarrow 12\ H_2 + 6\ CO_2 \qquad \text{Eqn 1.3}$$

1.1.1 Fermentative Bio-hydrogen Gas Production

1.1.1.1 Dark Fermentation

Dark fermentation is a promising way of using inexpensive feedstock, from several organic wastes as a substrate for hydrogen production. Usually, for fermentation processes monosaccharides are the preferred carbon source which can be produced by acidic or enzymatic hydrolysis of polysaccharides (starch, cellulose). During the breakdown of glucose by organisms to produce hydrogen gas, major products, principally volatile fatty acids (VFA) (acetic, butyric, propionic acids) and CO_2 are also produced. Theoretically, a maximum of 4 mol of H_2 can be produced per mole glucose when acetic acid is the only VFA product, when butyric acid is the only end- product, 2 mol of H_2 per mole of glucose is obtained as shown in equations 1.4 and 1.5, respectively. Propionic acid formation consumes 1 mol of H_2 per mole of propionic acid (Argun & Kargi, 2011). However, practically, lower yields are obtained since part of the glucose is consumed for microbial growth, maintenance and formation of a mixture of VFAs. (Argun, Kargi, & Kapdan, 2009b).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \Delta G^o = -206\ kJ \qquad \text{Eqn 1.4}$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_2CH_2CH_2COOH + 2CO_2 + 2H_2 \qquad \text{Eqn 1.5}$$

Hydrogen gas production by dark fermentation is carried out by various types of microorganisms, under anaerobic conditions. Bacteria known to produce hydrogen gas include species of spore forming strict anaerobic *Clostridia* (*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*) (Kapdan & Kargi, 2006).

Great deal of research was conducted to improve fermentative hydrogen production efficiency. In order to achieve high hydrogen yields, various parameters have to be controlled during the fermentation process such as inoculum, substrate,

reactor type, metal ion and environmental conditions (pH, temperature, ORP). pH has been considered to be one of the most important parameters affecting metabolic pathway, hydrogen yield and specific production rate. Argun & Kargi, (2011) reported that optimal pH value is between 5.5 and 6.5 since fermentative hydrogen production occurs in acidogenic phase of anaerobic metabolism. Optimum temperature for dark fermentation varies depending on the type of bacteria: mesophilic (25–40 °C), thermophilic (40–65 °C), extreme thermophilic (65–80 °C) and hyper-thermophilic (T >80 °C) fermentations are possible (Levin & Chahine, 2010). Since hydrogenase enzyme activity is strictly sensitive to oxygen, the ORP of the fermentation medium has to be kept below -150 mV.

1.1.1.2 Photo Fermentation

Photosynthetic bacteria (also known purple non-sulphur bacteria (PNS)) utilize a wide variety of volatile fatty acids (VFA) as electron donors and light as energy source to produce hydrogen in the presence of nitrogenase enzyme. As shown in Eqn. 1.6 the maximum theoretical yield is 4 mol of hydrogen per mol of acetic acid.

$$CH_3COOH + 2\ H_2O + \text{light energy} \rightarrow 4\ H_2 + 2\ CO_2 \qquad \text{Eqn 1.6}$$

Rhodopseudomonas capsulata, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodopseudomonas palustris are some of the well-known PNS bacteria. For efficient hydrogen production, photo-fermentation bacteria require complex nutrients (EDTA, Mo, Fe), strict control of environmental conditions (T = 30–35 °C, pH = 6.8–7.5), non-inhibitory concentrations of VFAs (< 2500 mg L^{-1}) and NH_4–N (< 50 mg L^{-1}) (Argun & Kargi, 2010c). Another important factor is also the type of light source and the light intensity. Beside sun light, different kind of light sources were used for photo-fermentation. Argun & Kargi (2010a) found that halogen lamp is the most suitable light source as compared to tungsten, infrared, fluorescent, sun light.

1.1.1.3 Combined Fermentation

Dark and photo-fermentations can be used in sequential or combined modes. In most of the reported studies, sequential batch fermentations were used for bio-hydrogen production where the effluent of dark fermentation was used for H_2 production by photo-fermentation after some pre-treatment. Combined dark-light fermentations for bio-hydrogen production have considerable advantages over sequential fermentation due to reduced fermentation time and high hydrogen yields. Theoretically, as shown Eqn.1.7, 1 mol of glucose can be converted to 12 mol of hydrogen if the only VFA is acetic acid. Operating conditions in combined fermentation was recommended to be closer to that of the photo-fermentation (pH = 7–7.5, ORP = −150 mV, 30 °C) rather than dark fermentation since PNS bacteria are known to be more sensitive to changes in environmental conditions (Argun & Kargi 2010c). The major problem in the combined fermentation is the lower hydrogen formation rates, once PNS bacteria are adapted to carbohydrate utilization first and VFAs later, it takes a long lag time in between (Argun & Kargi, 2010c; Ozmihci & Kargi, 2010a; Sagnak & Kargi, 2011)

$$C_6H_{12}O_6 + 6\,H_2O + \text{light energy} \rightarrow 12\,H_2 + 6\,CO_2 \qquad \text{Egn.1.7}$$

1.2 Literature Review

Variety of waste biomass such as barley straw, barley grain, corn stalk, corn grain, sugar beet, ground wheat, sugarcane bagasse have been used as raw materials for bio-hydrogen production (Argun, Kargi, Kapdan, & Oztekin, 2008a, 2008b, 2009a, 2009b; Cao et. al, 2009; Fan et. al, 2008; Guo et. al, 2010; Lin, Chang, & Hung, 2008). Hydrogen gas production from wastewater and solid wastes by dark fermentation has also been investigated (Dong, Zhenhong, Yongming, Xiaoying & Yu, 2009; Han & Shin, 2004; Gilroyed, Chang, Chu, & Hao, 2008; Kyazze et. al, 2008; Sivaramakrishna, Sreekanth, Himabindu & Anjaneyulu, 2009; Thong et. al, 2008).

Effects of nutrient concentrations (C, N, P, Fe) on bio-hydrogen production from wheat starch have been investigated by dark fermentation (Argun et. al, 2008a;

Oztekin, Kapdan, Kargi & Argun, 2008; Pan, Fan, Xing, Hou & Zhang, 2008). Heat pre-treatment of anaerobic sludge was found to be the most suitable method for dark fermentation (Argun, & Kargi, 2009)

Although most of the dark fermentation studies were carried out by batch system, continuous operations were also reported using different raw materials and microbial consortia for bio-hydrogen production (Azbar, Dokgoz, Keskin, Korkmaz & Syed, 2009; Cheng et.al, 2008; Hamilton et. al, 2010; Krupp & Widmann, 2008; Lee, Lin, Fangchiang & Chang, 2007; Ren, Li, Li, Wang & Liu, 2006; Van Ginkel & Logan, 2003; Van Ginkel, Oh & Logan, 2005; Zhang, Show, Tay, Liang & Lee, 2008)

Most of the photo and combined fermentation studies were performed using batch operation (Argun, Kargi, & Kapdan, 2009c; Arooj, Hun, Kim, Kim & Shin, 2008; Asada et. al, 2006; Chen et.al., 2008; Ding et.al, 2009; Hussy, Hawkes, Dinsdale, & Hawkes, 2003; Hawkes., Hussy, Kyazze., Dinsdale, & Hawkes, 2007; Koku, Eroglu, Gunduz, Yucel & Turker, 2003; Liu et. al, 2010; Ozmihci, & Kargi, 2010c; Shi & Yu, 2004; Shi & Yu, 2005; Sun et al, 2010; Xie et. al, 2010; Yokoi, Tokushige, Hirose, Hayashi & Takasaki,1998).There is limited number of continuous studies on photo and combined fermentation (Argun & Kargi, 2010c; Fascetti, D'addario, Todini & Robertiello,1998; Fascetti, & Todini, 1995; Hoekema, Bijmans, Janssen, Tramper & Wijffels, 2002; Jeong, Cha, Yoo, & Kim, 2007; Najafpour, Younesi, & Mohamed, 2003; Ozmihci & Kargi, 2010b; Tsygankov, Hirata, Miyake, Asada & Miyake, 1994).

Hydrogen production using heat treated digested sewage sludge from particulate wheat starch (7.5 g L^{-1} total hexose) was investigated at 18 and 12 hour hydraulic retention times (HRT), pH 4.5 and 5.2, and temperatures 30 ^{o}C and 35 ^{o}C (Hussy et al. 2003). It was reported that reduction of HRT to 12 hour and sparging with nitrogen gas resulted in more suitable operation and improved hydrogen yield from 1.3 to1.9 mol H_2 mol^{-1} hexose (Hussy et. al, 2003). The same authors also investigated continuous hydrogen production from refined sucrose, pulped sugar-beet and a water extract of sugar-beet with a simple batch start-up procedure at pH 5.2

and 32°C, using anaerobic sewage sludge. Daily hydrogen yields were 1.0 ± 0.1 and 0.9 ± 0.2 mol mol^{-1} hexose with 14–15 h retention time and 16 kg total sugar m^{-3} d^{-1} organic loading rate, for refined sucrose and pulped sugar-beet, respectively. With nitrogen sparging hydrogen yields were 1.9 ± 0.2 and 1.7 ± 0.2 mol mol^{-1} hexose for refined sucrose and water extract of sugar-beet, respectively (Hussy, Hawkes, Dinsdale, & Hawkes, 2005).

Arooj et. al, (2008) performed a CSTR using corn starch for dark fermentative hydrogen gas production. Anaerobic digested sludge was used as inoculum. Maximum hydrogen yield was 0.92 mol H_2 mol^{-1} glucose at HRT 12 h. The highest HPR and SHPR were reported as 5.59 L H_2 L^{-1} d^{-1} and 2.98 L H_2 g^{-1} VSS d^{-1}, respectively at HRT 6 h, (Arooj et al. 2008).

Chen et.al. (2008) reported hydrogen production from hydrolyzed starch using *Clostridium butyricum* CGS2 at different HRTs. When the HRT was shortened from 12 to 2 h, the specific hydrogen production rate increased from 250 to 534 ml g^{-1} VSS h^{-1}, while the hydrogen yield decreased from 2.03 to 1.50 mol H_2 mol^{-1} glucose. The volumetric H_2 production rate reached a high level of 1.5 L h^{-1} L^{-1} while operating at 2 h HRT (Chen et.al., 2008).

Li et. al. (2010) operated two CSTRs for dark fermentation. One reactor was fed with 12 g L^{-1} starch and 8 g L^{-1} peptone (SP) while the other one 12 g L^{-1} glucose and 8 g L^{-1} peptone (GP) with a working volume of 10 L and 1.5 L, respectively. The highest hydrogen yields for GP and SP reactors were 1.55 and 1.02 mmol H_2 $mmol^{-1}$ hexose, at 6 h and 3 h HRTs, respectively. The maximum hydrogen production rates for GP and SP reactors were 1247 and 412 mmol H_2 L^{-1} d^{-1} at HRTs of 2 and 3 h, respectively (Li et.al., 2010).

Argun & Kargi (2010c) investigated continuous combined dark and light fermentation in a hybrid annular bioreactor. Effects of HRT on hydrogen formation yield and rate were studied. *Clostridium beijerinkii* DSM 791 and *R. sphaeroides*-RV were used as microbial strains with a biomass ratio of C/R = 1/3.9. Boiled waste

ground wheat containing 5 g L^{-1} wheat starch was used as the feed substrate with different loading rates depending on the HRT. The system was operated under 10 klux illumination with halogen and fluorescent lamps. pH and temperature were kept around 7–7.5 and 32 ± 2 °C, respectively. Hydrogen yields, SHPR and VHPR at steady-state were 0.6 mol H_2 mol^{-1} glucose, 9.16 mL H_2 g^{-1} h^{-1}, 5.95 mL H_2 L^{-1} h^{-1}, respectively at HRTs = 6 days, 1 day, 1 day. (Argun & Kargi, 2010).

Hydrogen gas was produced from starch by combined fermentations operated in fed-batch mode by Yokoi et.al. (1998). *Rhodobacter* sp. M-19 and *C. butyricum* were used as inoculum cultures with an initial biomass ratio of *R*/*C* = 10/1. The fermenter containing 1 g L^{-1} starch was fed with 1 mL of 50 g L^{-1} starch solution at 24 h intervals for four times. pH, temperature and light intensity were 6.8, 30 °C and 5 klux, respectively. The system resulted in a high yield of 6.6 mol H_2 mol^{-1} glucose (Yokoi et.al., 1998).

1.2 Objectives and the Scope

The major objective of this study is to investigate bio-hydrogen gas production from acid hydrolyzed wheat starch (AHWS) by batch and continuous dark and photo-fermentations.

Detailed objectives of the study can be summarized as follows:

- To investigate the effects of initial substrate (AHWS) and biomass concentration on bio-hydrogen gas production rate and yield in batch dark fermentation.

- To investigate the effects of hydraulic residence time (HRT) on bio-hydrogen gas production rate and yield in continuous dark fermentation.

- To investigate the effects of hydraulic residence time on bio-hydrogen gas production rate and yield in continuous photo-fermentation.

- To investigate the effects of hydraulic residence time feeding on bio-hydrogen gas production rate and yield in continuous combined fermentation.

- To determine the most suitable operating conditions maximizing hydrogen gas yield and formation rate.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Batch Dark Fermentation

2.1.1 Effects of Initial Wheat Powder Solution and Biomass Concentrations

2.1.1.1 Experimental Set Up and Procedure

Batch dark fermentation experiments were carried out in 0.5 L serum bottles (Isolab-Germany Boro 3.3) with 250 ml fermentation volume. Silicone rubber stoppers and screw caps were used to avoid gas leakage from the bottles. The ground wheat of 200 mesh was acidified to pH = 3 (adjusted by H_2SO_4) and hydrolyzed at 90 °C for 15 min in an autoclave. The solid phase of the hydrolyzate was separated by centrifugation at 8000 g and the supernatant sugar solution was neutralized to pH = 7 by addition of 10 M NaOH. Sugar solution from hydrolyzed wheat starch was nitrogen (N) and phosphorous (P) deficient. Nitrogen, phosphorus and Fe(II) were supplemented to yield N/P/Fe/C ratio of 2/0.8/1.5/100 by using urea (CON_2H_4), KH_2PO_4 and $FeSO_4$ as nitrogen, phosphorus and iron source, respectively. Initial pH of the medium was adjusted to 6.5. The oxidation-reduction potential (ORP) was adjusted to nearly −200 mV by addition of 100 mg L^{-1} cysteine HCl. Initial total sugar concentration was varied between 3.9 and 27.5 g L^{-1} at constant biomass concentration of 1.3 g L^{-1} in the first set of experiments. Biomass concentration was varied between 0.28 g L^{-1} and 1.38 g L^{-1} at initial total sugar concentration of 7.2 ± 0.2 g L^{-1} in the second set. The inoculated bottles were placed in an incubator at a constant temperature of 37 °C. The bottles were mixed manually several times a day.

2.1.1.2 Organisms

The anaerobic sludge was obtained from the acidogenic phase of anaerobic wastewater treatment plant of PAK MAYA Bakers Yeast Company in Izmir, Turkey. The culture was concentrated by sedimentation and was heated in boiling water for 5 h in order to select the spore forming acidogenic bacteria and to eliminate the

hydrogen consuming methanogens. The heat-treated anaerobic sludge was cultivated

in a synthetic media containing glucose (60 g L^{-1}), peptone (10 g L^{-1}), yeast extract (0.6 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.25 g L^{-1}), K_2HPO_4 (1 g L^{-1}), KH_2PO_4 (1 g L^{-1}), l-cysteine $HCl.H_2O$ (0.1 g L^{-1}) at 37 °C and pH = 6.8 in an incubator. Argon gas was passed through the cultivation media before incubation and the flasks were closed with gas-tight silicone stoppers and screw caps. The cultivated organisms were used for inoculation of experimental bottles after three days of incubation.

2.1.1.3 Analytical Methods

Samples removed from the liquid phase everyday were centrifuged at 8000 g and the clear supernatants were used for analysis of total sugar (TS) and total volatile fatty acids (TVFA). Total sugar concentrations were determined by the acid-phenol spectrometric method (Dubois et al., 1956). TVFA analyses were carried out by using analytical kits (Spectroquant, 1.01763. 0001, Merck, Darmstadt, Germany) and a PC spectrometer (WTW Photolab S12).

Hydrogen gas was sampled from the head space of the bottles by using gas-tight glass syringes. Hydrogen gas concentration in the gas phase was measured by using a gas chromatograph (HP Agilent 6890). The column was Alltech, Hayesep D 80/100 6" × 1/8" × 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):

$$V_{H2,\,i} = V_{H2,\,i\,-1} + V_W\ C_{H2,i} + V_{G,i}\ C_{H2,i} - V_{G,i-1}\ C_{H2,\,i-1} \qquad \text{Eqn 2.1}$$

where $V_{H2,\,i}$ and $V_{H2,\,i\,-1}$ are the volumes of cumulative hydrogen (mL) calculated

after the i^{th} 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 i^{th} 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 i^{th} 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 (cell) concentration in the inoculum was determined by filtering 20 ml sample through a 0.45 μm millipore filter, drying at 105 °C 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 Sci., Germany). pH of the medium decreased from an initial value of 6.5 to nearly 4.5 in early stages of fermentation due to VFA production and was adjusted to 7.0 by addition of 10 M NaOH twice a day. pH was maintained between 6.0 and 7.0 by manual pH control. ORP values varied between −100 and −300 mV, in general.

2.2 Continuous Fermentation

Experiments were carried out in sealed serum bottles (Isolab-Germany Boro 3.3). The bottles equipped with silicone rubber stoppers and screw caps and metal valves. Silicon tubing and peristaltic pumps were not used for feeding and effluent removal in continuous operation to avoid any hydrogen gas leakage. Instead, feed and effluent solutions were added and removed from the bottles periodically with the same rate using syringes.

Waste wheat was obtained from Soke Flour Co in Soke, Izmir, Turkey. The wheat particles were ground and sieved down to -200 mesh size in order to obtain the wheat powder. The wheat powder (WP) used as substrate for dark and photo-fermentations contained approximately 97% (w w_{-1}) starch and gluten, 3.4 mg g^{-1} total nitrogen and

1.72 mg g^{-1} phosphate-P. The stock solution (SS) contained 30 gL^{-1} WP and the pH was adjusted to 3.0 using concentrated H_2SO_4 and the solution was autoclaved at 121 ^oC for 30 minutes in order to hydrolyze wheat starch to glucose and maltose. The yield of starch conversion to soluble total sugar was 95% in acid hydrolysis. Hydrolyzed WP solution was centrifuged at 7000g to remove solids and the supernatant was neutralized to pH = 7 using 10 M NaOH solution.

In continuous photo-fermentation experiment effluent of batch dark fermentation of ground wheat starch was used as the feed solution.

Anaerobic conditions were maintained by passing argon gas from the head space of the bottles for 3 minutes at the beginning of the experiments. In all experiments, the initial oxidation reduction potentials (ORP) were around 50 ± 10 mV which decreased to -300 ± 50 mV at the end of the fermentation.

The reactors were operated continuously by periodic feeding and effluent removal. Samples were removed from the feed and the fermenter every day for TVFA, total sugar, NH_4-N, pH and oxidation-reduction potential (ORP) measurements before pH adjustments.

2.2.1 Continuous dark fermentation with periodic feeding

2.2.1.1 Experimental Set Up and Procedure

Dark fermentation of acid hydrolyzed ground wheat starch for bio-hydrogen production by periodic feeding and effluent removal was investigated at different feeding intervals. Experiments were carried out in 0.5 L serum bottles and with fermentation volume of 0.25 L of hydrolyzed starch solution containing nutrients and were inoculated with concentrated and heat treated anaerobic sludge. Serum bottles were placed on magnetic stirrers (100 rpm) and were heated to keep the temperature around 35 ± 2 °C. Feed solution contained mainly glucose and maltose with small amounts of non-hydrolyzed starch. Concentrated stock sugar solution was diluted properly to obtain 9 ± 0.5 g L^{-1} total sugar concentration in the feed and was placed on a magnetic stirrer (100 rpm) in a deep refrigerator at 4 °C in order to avoid

any decomposition. The feed solution was supplemented with 90 mg L^{-1} urea, 2.8 g L^{-1} K_2HPO_4, 3.9 g L^{-1} KH_2PO_4, 50 mg L^{-1} $MgSO_4.7H_2O$, 25 mg L^{-1} $FeSO_4.7H_2O$. The feed solution was added and removed from the bottles periodically with certain flow rates (1000–100 ml d^{-1}) to obtain desired HRTs between 6 and 60 hours. Total sugar loading rates were varied between 0.85 and 8.5 g total sugar d^{-1}. The reactors were operated batch-wise until carbohydrates were completely fermented before the start-up of continuous operation and were performed until hydrogen production rate (ml d^{-1}), effluent sugar and TVFA concentrations reached a steady level for three to four days for every operation. Argon gas was passed through the head space of the fermenter after every sampling period in order to remove hydrogen gas from the head space of the reactor and to sustain anaerobic conditions.

2.2.1.2 Organisms

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 culture was concentrated by sedimentation and was boiled for 1.5 h at pH 5.9 in order to select hydrogen producing, spore forming acidogenic bacteria and to eliminate hydrogen consuming methanogens. The heat treated anaerobic sludge was cooled and was used for inoculation of bottles.

2.2.1.3 Analytical Methods

The analytical methods used for continuous experiments were the same as in batch experiments as explained in part *2.1.1.3*.

2.2.2 Continuous photo-fermentation by periodic feeding

2.2.2.1 Experimental Set Up and Procedure.

Experiments were carried out in 0.5 L serum bottles with fermentation volume of 0.25 L which were placed on magnetic stirrers (100 rpm) in air conditioned room at 30 °C. Acid hydrolyzed wheat solution (AHWS) was diluted properly to obtain 10 g L^{-1} total sugar (starch + glucose) and was supplemented with 90 mg L^{-1} urea, 2.8 g L^{-1} K_2HPO_4, 3.9 g L^{-1} KH_2PO_4, 50 mg L^{-1} $MgSO_4.7H_2O$, 25 mg L^{-1}

$FeSO_4.7H_2O$ and was used for batch dark fermentation to produce dark fermentation effluent containing volatile fatty acids (VFAs). The effluent was centrifuged at 7000*g* for separation of the biomass, after 7 days of batch dark fermentation of acid hydrolyzed wheat starch. Dark fermentation effluent contained nearly 300 mg L^{-1} total sugar and 6 g L^{-1} TVFA. The supernatant was diluted to obtain the feed TVFA around 2 g L^{-1} and was supplemented with 20 mg L^{-1} EDTA, 50 μg L^{-1} $Na_2MoO_4.2H_2O$, 50 mg L^{-1} $MgSO_4.7H_2O$, 20 mg L^{-1} $FeSO_4.7H_2O$ + EDTA complex to be used as the photo-fermentation feed solution. Fermentation broth volume was kept constant at 0.25 L and hydraulic residence time (HRT) was varied by changing the daily feeding rate between 0.25 L d^{-1} (HRT =1 day) and 0.025 L d^{-1} (HRT = 10 day). TVFA loading rates were varied between 0.05 and 0.5 g TVFA d^{-1} . Bottles were illuminated from the opposite sides by using halogen lamps at 5 Klux light intensity.

2.2.2.2 Organisms

Pure *Rhodobacter sphaeroides* culture (NRRL B-1727) was obtained from USDA National Center for Agricultural Utilization Research, Peoria, IL, USA. *Rhodobacter* culture was grown on hydrogen gas production medium containing acetic acid (2 g L^{-1}), butyric acid (1 g L^{-1}), K_2HPO_4 (2.8 g L^{-1}), KH_2PO_4 (3.9 g L^{-1}), yeast Extract (0.5 g L^{-1}), $Na_2MO_4.2H_2O$ (0.75 mg L^{-1}), Na Glutamate (1.873 g L^{-1}), $MgSO_4.7H_2O$ (0.25 g L^{-1}), $FeSO_4.7H_2O$ + EDTA complex (10 mg L^{-1}) at pH 7.0 ± 0.2. The organisms were grown for five days at 32 °C under 5 Klux illumination using halogen lamps. The harvested cells (50 ml) were added to 200 ml of photo-fermentation media in the experimental bottles for 7 days of batch photo-fermentation before starting the continuous operation by periodic feeding.

2.2.2.3 Analytical Methods

The analytical methods used for continuous experiments were the same as in batch experiments as explained in part *2.1.1.3*. NH_4-N was determined by using analytical kits (Spectroquant NH_4-N 1.14752.0001,Germany) and a PC spectrometer (WTW Photolab S12). Total nitrogen was measured according to Standard

Methods (Greenberg et al., 2005) . 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.

2.2.3 Continuous combined fermentation by periodic feeding

2.2.3.1 Experimental set up and procedure

Experiments were carried out using 0.25 L sealed serum bottles with 0.15 L fermentation volume. The bottles were illuminated with halogen lamps at 5000 lux from the outer surface and were placed on magnetic stirrers (100 rpm) and in an air conditioned room at 30 ± 1 ^{o}C. Concentrated stock sugar solution was diluted properly to obtain 5.1 ± 0.1 g L^{-1} total sugar concentration in the feed and was placed on a magnetic stirrer (100 rpm) in a deep refrigerator at 4 ^{o}C in order to avoid any decomposition. The feed solution was supplemented with 0.25 gL^{-1} $MgCl_2$ $2H_2O$, 2.8 gL^{-1} K_2HPO_4, 3.9 gL^{-1} KH_2PO_4, 20 mgL^{-1} $FeSO_4$ $7H_2O$ (from $FeSO_4$. $7H_2O$ - EDTA complex), 50 μgL^{-1} $Na_2MoO_4.2H_2O$. Serum bottles were filled with 0.15 L feed solution and inoculated with a mixture of heat treated anaerobic sludge (dark fermentation bacteria, D) and *Rhodobacter sphaeroides* (light fermentation bacteria-L) with a D/L biomass ratio of 1/3. The initial dark and light fermentation bacteria concentrations were X_D = 0.137 gL^{-1} , X_L=0.410 gL^{-1} X_T = 0.547 g L^{-1}. By changing the frequency of feeding, hydraulic residence time (HRT) were varied between 1 and 8 days. Total sugar loading rates varied between 0.094 and 0.843 g total sugar d^{-1}. Experiments were performed until hydrogen production rate (ml d^{-1}) and effluent sugar concentrations reached a steady level for three to four days which were accepted as a steady state data. Before starting continuous operation the serum bottles were operated batch-wise for three days.

2.2.3.2 Organisms

The anaerobic organism was the same as in section *2.2.1.2* and was cultivated in medium containing glucose (60 gL^{-1}), peptone (10 gL^{-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}) for three days before inoculation of the experimental bottles.

The photo fermentation bacteria was the same as in section *2.2.2.2* .The culture was grown in a medium containing acetic acid (3 gL^{-1}), butyric acid (3 gL^{-1}), K_2HPO_4 (2.8 gL^{-1}), KH_2PO_4 (3.9 gL^{-1}), Yeast Extract (0.5 gL^{-1}) , $Na_2MoO_4.2H_2O$ (0.75 μgL^{-1}), Na-glutamate (1.873 gL^{-1}), $MgSO_4.7H_2O$ (0.25 gL^{-1}), $FeSO_4.7H_2O$ + EDTA complex (10 mgL^{-1}) and EDTA (20 mgL^{-1}) at pH 7.0. The culture was cultivated at for five days at 32 ^oC under 5000 lux illumination using halogen lamps. At the end of cultivation period, biomass concentrations in dark and photo-fermentation media were determined and the serum bottles were inoculated to yield dark and light-fermentation bacteria concentrations of X_D = 0.137 gL^{-1} and X_L = 0.410 gL^{-1} yielding X_D/X_L = 1/3.

2.2.3.3 Analytic Methods

The analytical methods used for continuous experiments were the same as in batch experiments as explained in part *2.1.1.3*. Light intensities and irradiation were measured using a light meter LX-1108 LT Lutron (LT Lutron, Taiwan) and an Apogee Pyronometer Sensor- PYR-P 357 (Apogee, USA), respectively.

2.3 Calculation methods

2.3.1 Calculations for batch dark fermentation

The generalized gas equation presented in Eqn 2.2 was used to calculate the mole number of cumulative hydrogen.

$$PV = nRT \qquad \text{Eqn 2.2}$$

where: n is 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 2.3 and the constants were determined by regression analysis with Statistica 5. The Gompertz equation has the following form (Han & Shin, 2004):

$$H(t) = P\exp\left\{ -\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\}$$ Eqn. 2.3

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.

Hydrogen formation yield and specific hydrogen production rate (SHPR) are important parameters indicating the effectiveness of fermentation. The yield was calculated by using the following equation.

$$Y = CHF / V_0 (S_0 - S)$$ Eqn. 2.4

where Y is the hydrogen gas yield (ml H_2 g^{-1} TS or mol H_2 mol^{-1} glucose); CHF is the cumulative hydrogen gas formation (mL); V_o is the initial fermentation volume (L); S_0 and S are the initial and final total sugar concentrations (g L^{-1}).

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

$$R_x = R_m / V_o X_o$$ Eqn 2.5

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}).

2.3.1.1 Mathematical model

Since H_2 gas is a growth associated product, specific hydrogen gas production rates (SHPR) obtained at different substrate concentrations were correlated with the

Briggs–Haldane equation describing substrate inhibition at high substrate concentrations in equation.

$$R_{H_2} = \frac{k \times X_0 \times S_0}{K_s + S_0 + \frac{S_0^2}{K_{SI}}} \qquad \text{Eqn.2.6}$$

where, R_{H2} is the initial volumetric rate of hydrogen gas formation (ml H_2 L^{-1} h^{-1}); k is the specific hydrogen gas formation rate constant (ml H_2 g^{-1} cell h^{-1}); X_o is the initial biomass concentration (g L^{-1}); S_o is the initial total sugar concentration (g L^{-1}); K_s is the saturation constant (g L^{-1}); K_{SI} is the substrate (total sugar) inhibition constant. In terms of specific rate (R_x), equation 3.6 can be written as follows,

$$R_x = \frac{R_{H_2}}{X_0} = \frac{k \times S_0}{K_s + S_0 + \frac{S_0^2}{K_{SI}}} \qquad \text{Eqn.2.7}$$

where R_x is the initial specific rate of hydrogen formation (SHPR, ml H_2 g^{-1} cell h^{-1}).

2.3.2 Calculations 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 2.1 in section *2.1.1.3*. The mole number of hydrogen was calculated as explained in Eqn 2.2

Steady-state volumetric (R_v, ml H_2 L^{-1} d^{-1}) and specific (R_x, ml H_2 g^{-1} biomass d^{-1}) rates of hydrogen gas formation were calculated by using the equations 3.5 and 3.6, respectively.

$$R_v = V_{H2} / V \qquad \text{Eqn 2.8}$$

and

$$R_x = V_{H2} / X V \qquad \text{Eqn.2.9}$$

where V_{H2} is daily hydrogen gas production (ml H_2 d^{-1}); V is the volume of fermentation broth; X is the biomass concentration in the fermenter at the steady-state (g L^{-1}).

Hydrogen yields at the steady-state of every continuous operation was calculated by using the following equation

$$Y_{H2} = \frac{V_{H_2}}{Q\,(S_0 - S)} \qquad \text{Eqn.2.10}$$

where V_{H2} is daily hydrogen gas production (ml H_2 d^{-1}); Q is the feed flow rate (L d^{-1}) ; S_o and S are the feed and effluent total sugar concentrations (g L^{-1}) and Y_{H2} is the hydrogen yield (ml H_2 g^{-1} total sugar).

The daily substrate loading rate (g starch d^{-1}) was calculated by multiplying the initial feeding substrate concentration with the flow rate (Q S_0). The substrate loading rate can calculated by dividing the daily substrate loading rate to the volume of reactor, or dividing the substrate concentration to the HRT or multiplying dilution rate (D = Q/ V) with initial substrate concentration as shown in the following equation;

$$L_s = Q\,S_0 / V = S_0 / \mathrm{HRT} = D\,S_0 \qquad \text{Eqn.2.11}$$

The growth yield coefficient was calculated by dividing the steady state biomass concentration to the consumed substrate concentration as shown in equation 2.3.11.

$$Y_{x/s} = X / (S_0 - S) \qquad \text{Eqn.2.12}$$

CHAPTER THREE
RESULTS AND DISCUSSION

3.1 Batch Dark Fermentations

3.1.1 Effects of initial total sugar concentration

Figure 4.1 depicts variation of cumulative hydrogen gas formation (CHF) with time for different initial total sugar concentrations. Cumulative hydrogen gas volumes increased with time and reached the final level within 41 h for all substrate concentrations. High substrate concentrations yielded higher cumulative hydrogen gas volumes. The lowest CHF (137 ml) was obtained with the lowest total sugar concentration of (3.9 g L^{-1}) and the highest CHF (696 ml) was realized with the highest total sugar of 27.5 g L^{-1}. CHF in control bottles was less than 30 ml which was probably due to contamination.

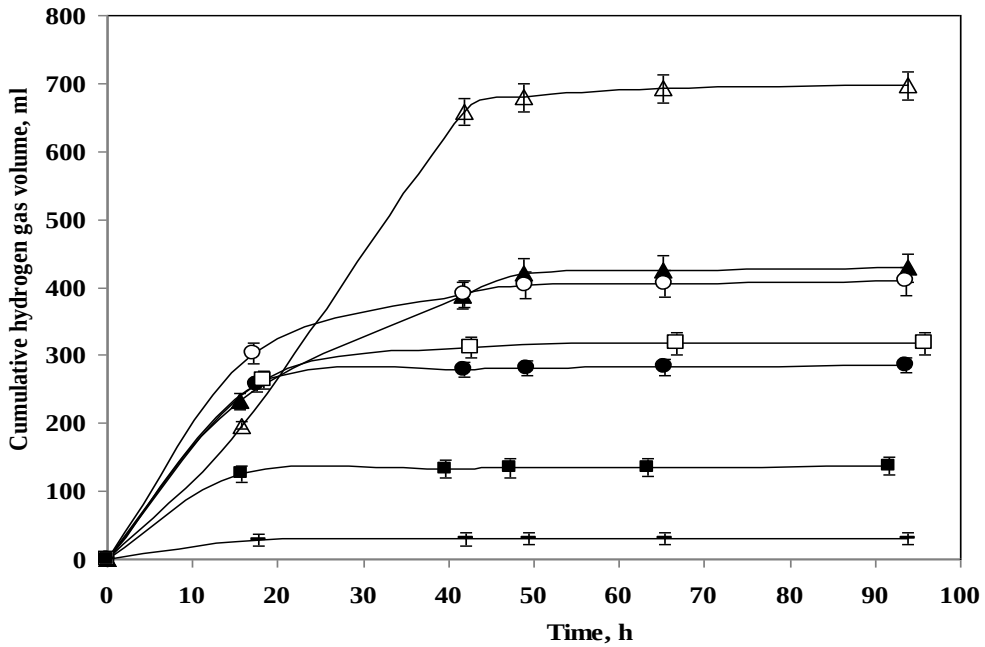

Figure 3.1 Variations of cumulative hydrogen gas formation with time for different initial total sugar concentrations. Initial biomass: X_o = 1.3 g L^{-1}. Total sugar concentrations (g L^{-1}): (■ 3.9, □ 7.6, ● 10, ○ 15, ▲ 22, Δ 27, —— control).

Cumulative hydrogen formation data presented in Figure 3.1 were correlated with the Gompertz equation and the constants were determined by regression analysis using the STATISTICA 5.0 program as explained in section 3.1.

Table 3.1 Gompertz equation constants for variable initial total sugar concentrations. X_o = 1.3 g L^{-1}.

Initial substrate (TS) concentration (g L^{-1})	***P* (ml)**	***R_m* (ml h^{-1})**	**λ (h)**	***R^2***
3.9	135.1	14.9	3.5	0.999
7.6	316.4	22.3	4.3	0.992
10.0	281.9	26.1	4.0	0.999
15.0	402.7	25.1	3.8	0.999
22.0	420.8	18.3	2.9	0.997
27.5	696.2	30.5	9.3	0.996

Gompertz equation constants for different initial substrate concentrations are presented in Table 3.1. Hydrogen production potential increased with increasing initial total sugar concentration as expected. The potential hydrogen gas production was 135 ml for 3.9 g L^{-1} total sugar (TS) concentration which increased to 420 ml for TS = 22 g L^{-1}. The highest hydrogen gas production potential of 696 ml was obtained at the highest total sugar concentration of 27.5 g L^{-1}. The maximum hydrogen production rates (HPR, R_m) calculated from the Gompertz equation also increased from 14.9 ml h^{-1} to 26.1 ml h^{-1} when total sugar was increased from 3.9 g L^{-1} to 10.0 g L^{-1}. The highest HPR (30.5 ml h^{-1}) was obtained with the highest substrate concentration of 27.5 g L^{-1}. The lag phases for most of the substrate concentrations were relatively short (λ = 3–4 h) due to fermentation of readily available soluble sugar compounds derived from acid hydrolysis of wheat starch.

Variations of final sugar and TVFA concentrations with the initial total sugar contents are presented in Table 3.2. Final TVFA concentrations increased with increasing initial total sugar concentrations as expected. Percent substrate utilization increased from 91.6% to 98.6% when initial total sugar was increased from 3.9 to

15 g L^{-1} (Table 3.2). Further increases in total sugar concentration resulted in slight decreases in percent sugar utilization. Sugar compounds were converted to H_2, VFAs, CO_2 and biomass during dark fermentation. Final TVFA concentrations are closely related to hydrogen gas production since H_2 gas is a co-product of VFA formation. High final TVFA concentrations yielded high CHFs. The final TVFA concentration was 2.035 g L^{-1} at the lowest total sugar of 3.9 g L^{-1} which steadily increased and reached 5.935 g L^{-1} at initial total sugar of 27.5 g L^{-1}. Percent conversion of sugars to TVFA was 53% for the lowest TS concentration of 3.9 g L^{-1} which decreased to 30% and 21% for initial total sugar concentrations of 10 g L^{-1} and 27.5 g L^{-1} respectively, indicating product (VFA) inhibition at high initial sugar concentrations.

Table 3.2 Variation of total percent sugar utilization and TVFA formation with initial substrate concentration. X_o = 1.3 g L^{-1}.

Initial total sugar, TS_o (g L^{-1})	**Final total sugar TS_f (g L^{-1})**	**Percent substrate utilization**	**Initial TVFA concentration (g L^{-1})**	**Final TVFA concentration (g L^{-1})**	**Percent substrate conversion to TVFA**
3.9	0.33	91.6	0.134	2.035	53.0
7.6	0.43	94.4	0.406	3.13	37.9
10.0	0.206	97.9	0.147	3.075	30.1
15.0	0.216	98.6	0.153	4.410	28.8
22.0	1.09	95.0	0.298	6.205	28.3
27.6	1.05	96.2	0.279	5.935	21.3

Specific hydrogen production rates (SHPR, ml H_2 g^{-1} cell h^{-1} at 37 °C, 1atm) were calculated by using the equations 3.4 as explained in section 3.1. Variation of specific rate of hydrogen production (SHPR) with initial substrate concentration is depicted in Figure 3.2. The rate increased from 33 ml H_2 g^{-1} cell h^{-1} to 83 ml g^{-1} cell h^{-1} with the increase in substrate concentration from 3.9 g L^{-1} to 10 g L^{-1}. However, a sharp decrease in SHPR to 58 ml H_2 g^{-1} cell h^{-1} was observed at substrate concentrations above 15 g L^{-1} indicating product inhibition caused by high TVFA

concentrations. The optimum initial total sugar concentration yielding the highest SHPR was found to be around 10 g L^{-1}.

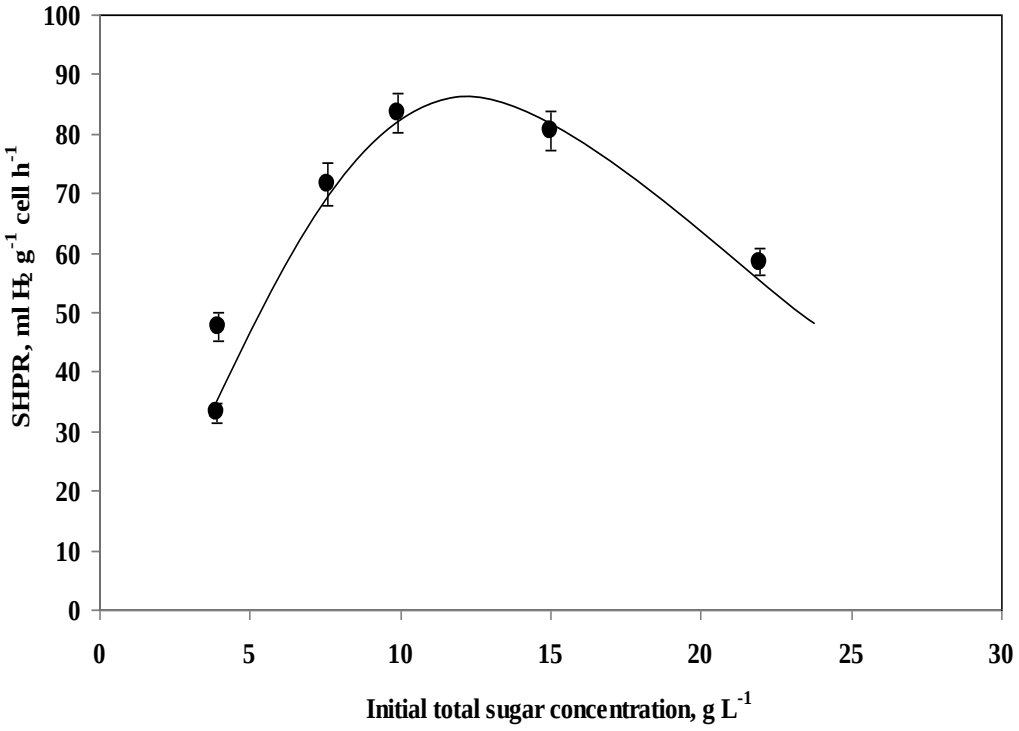

Figure 3.2 Variation of specific hydrogen gas production rate (SHPR) with initial total sugar concentration. X_o = 1.3 g L^{-1}.

The SHPR obtained at different initial substrate concentrations were used to determine the kinetic coefficients of equation 3.7. in section 3.1. Non linear regression analysis using the STATISTICA 5.0 program resulted in the following kinetic constants: k = 204.0 ml H_2 g^{-1} cell h^{-1}, K_S = 9.7 g L^{-1}, and K_{SI} = 13.4 g L^{-1}.

Equation 3.7 in section 3.1 takes the following form with the determined constants:

$$R_x = \frac{R_{H_2}}{X_0} = \frac{204.0 \times S_0}{9.7 + S_0 + \frac{S_0^2}{13.4}} \qquad \text{Eqn.3.1}$$

The observed and predicted SHPRs at different substrate concentrations were in good agreement (R2 = 0.94). The substrate concentration resulting in the maximum

rate was determined as Smax = 11.4 g L−1 by using the following equation;

$$S_{max} = \sqrt{K_S \times K_{SI}} \qquad \text{Eqn.3.2}$$

S_{max} value determined from equation 3.2 is in agreement with the experimental results (Fig. 3.2) indicating goodness of the fit of the kinetic constants.

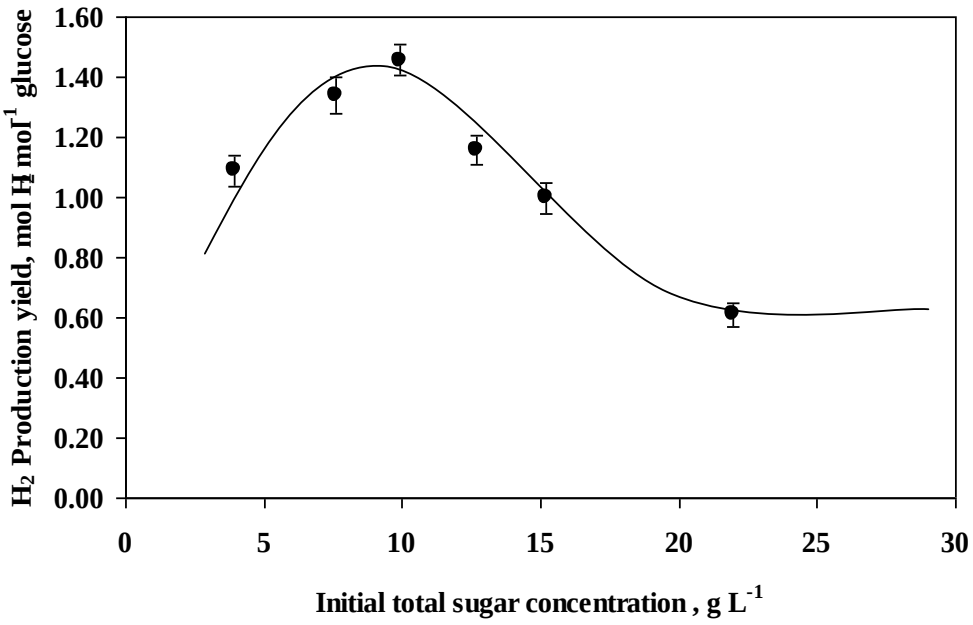

Figure 3.3 Variation of hydrogen gas yield with initial total sugar concentration. X_o = 1.3 g L^{-1}.

The yield of hydrogen gas formation is an important parameter determining the effectiveness of fermentation. The yield was calculated by using the equation 2.4 in section 2.3.1 Variation of hydrogen gas yield with the initial substrate concentration is depicted in Figure 3.3. Hydrogen yield increased to Y = 1.46 mol H_2 mol^{-1} glucose when total sugar concentration was increased from 3.9 g L^{-1} to 10 g L^{-1} (Figure 3.3). However, further increases in the substrate concentration resulted in decreases in the yield mainly due to product inhibition at high TVFA concentrations. The optimum initial total sugar concentration was 10 g L^{-1} with a hydrogen gas yield of 1.46 mol H_2 mol^{-1} glucose.

3.1.2 Effects of initial biomass concentration

Figure 3.4 depicts variation of cumulative hydrogen gas volume with time for different initial biomass (cell) concentrations. Cumulative hydrogen gas formation (CHF) did not vary substantially with the initial cell concentration while it was proportional with the initial substrate concentration. Biomass (cell) concentration mainly affected the rate of H_2 gas formation. Most of the H_2 gas formation was realized within 20 h of fermentation period. CHFs were between 300 and 320 ml for biomass concentrations between 0.55 g L^{-1} and 1.38 g L^{-1}. The highest CHF (386 ml) was obtained with the lowest biomass concentration of 0.28 g L^{-1}. Low CHF at high biomass concentrations may be due to bacterial floc formation at high cell concentrations causing substrate diffusion limitations.

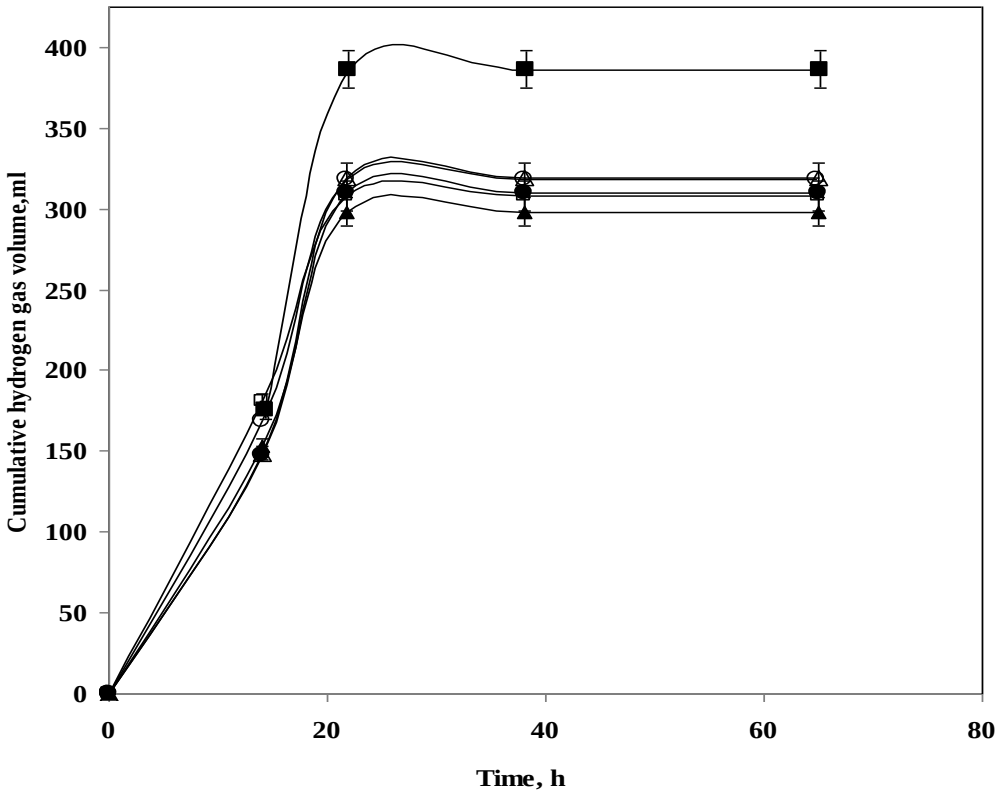

Figure 3.4 Variation of cumulative hydrogen gas formation (CHF) with time for different initial biomass concentrations. S_o = 7.2 ± 0.2 g L^{-1}. Biomass concentration (g L^{-1}): ■ 0.28, □ 0.55, ▲ 0.72, Δ 0.83, ● 1.10, ○ 1.38.

Gompertz equation constants were in agreement with the experimental results. Increases in biomass concentration resulted in slight decreases in hydrogen gas volume (Table 3.3). The highest H_2 gas production potential (387 ml) was obtained with the lowest biomass concentration while the lowest H_2 volume (271 ml) was realized at the biomass concentration of 0.72 g L^{-1}. The lag periods were around λ = 12 h for all initial biomass concentrations.

Table 3.3 Gompertz equation constants for variable initial biomass concentration. S_o = 7.2 g L^{-1}.

Biomass Con. (g L^{-1})	***P* (ml)**	***R_m* (ml h^{-1})**	**λ (*h*)**	***R^2***
0.28	387.3	92.4	12.5	0.999
0.55	306.7	63.7	11.2	0.999
0.72	271.4	59.4	8.1	0.913
0.83	319.8	79.8	12.2	0.999
1.10	309.7	98.9	12.6	1.000
1.38	319.1	68.8	11.6	0.999

Effects of biomass (cell) concentration on substrate utilization and final TVFA concentrations are summarized in Table 3.4. Substrate utilization and TVFA production were almost independent from biomass concentration. The effluent total sugar concentration was around 200 mg L^{-1} with 97% substrate utilization. High substrate utilization indicated that the anaerobic bacteria were active throughout the fermentation. A slight increase in the final TVFA concentration from 4350 mg L^{-1} to 5090 mg L^{-1} was observed with the increase in biomass concentration from 0.28 g L^{-1} to 1.38 g L^{-1}. Percent substrate conversion varied between 50 and 61% depending on initial biomass concentration. The highest percent conversion (60.9%) was obtained with the highest biomass concentration.

Table 3.4 Variation of substrate utilization and TVFA formation with initial biomass concentration.Initial TS = 7.2 ± 0.2 g L^{-1}.

Biomass Concent. (g L^{-1})	**Initial total sugar, TS_o (mg L^{-1})**	**Final total sugar, TS_f (mg L^{-1})**	**Percent substrate utilization**	**Initial TVFA concent (mg $L^{-1)}$**	**Final TVFA (mgL^{-1})**	**Percent substrate conversion to TVFA**
0.28	6860	173	97.5	307	4350	60.5
0.55	7229	209	97.1	512	4565	57.7
0.72	7343	200	97.3	663	4690	56.4
0.83	7598	200	97.4	629	4390	50.8
1.10	7371	212	97.1	909	4570	51.1
1.38	7116	293	95.9	934	5090	60.9

Fig. 3.5 presents the effect of biomass (cell) concentration on specific hydrogen production rate (SHPR). SHPRs were calculated by using equation 2.5 in section 2.3.1. The highest SHPR (1221 ml H_2 g^{-1} cell h^{-1}) was obtained with the lowest biomass concentration 0.28 g L^{-1}. SHPRs decreased with increasing initial biomass concentration as predicted by equation 3.4 in section 3.1 yielding the lowest SHPR (181 ml H_2 g^{-1} cell h^{-1}) at the highest biomass concentration of 1.3 g L^{-1}. The optimum biomass concentration resulting in the highest SHPR was 0.28 g L^{-1}. Similar trend was observed for hydrogen gas yield which decreased with increasing biomass concentration. The highest hydrogen yield (1.52 mol H_2 mol^{-1} glucose) was obtained with the lowest biomass concentration of 0.28 g L^{-1} (Fig. 4.6). Further increases in biomass concentration resulted in lower hydrogen yields of 1.14 mol H_2 mol^{-1} glucose for biomass concentrations between 0.5 g L^{-1} and 1.3 g L^{-1}.

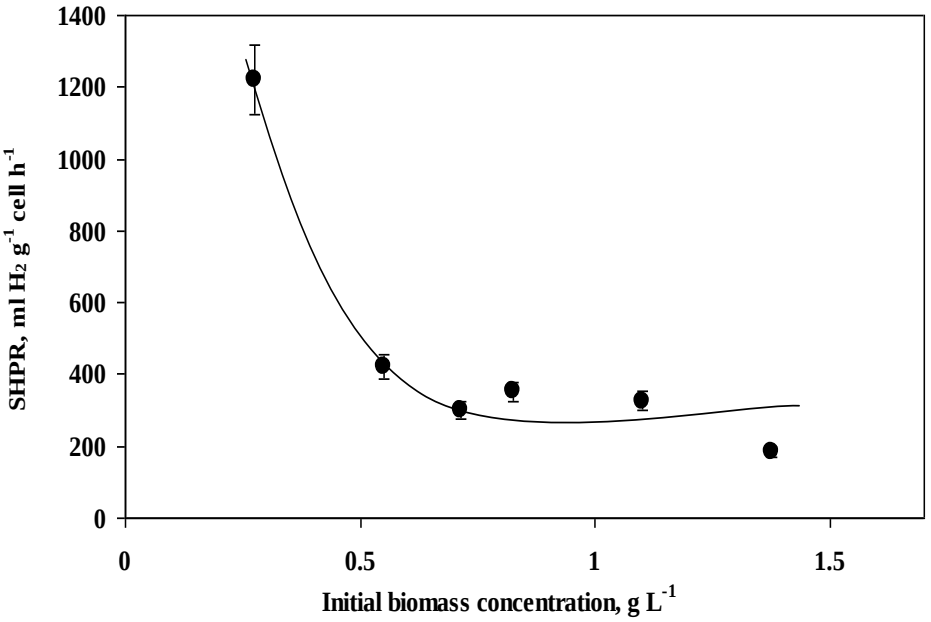

Figure 3.5 Variation of specific hydrogen gas production rate with initial biomass concentration. S_o = 7.2 ± 0.2 g L^{-1}.

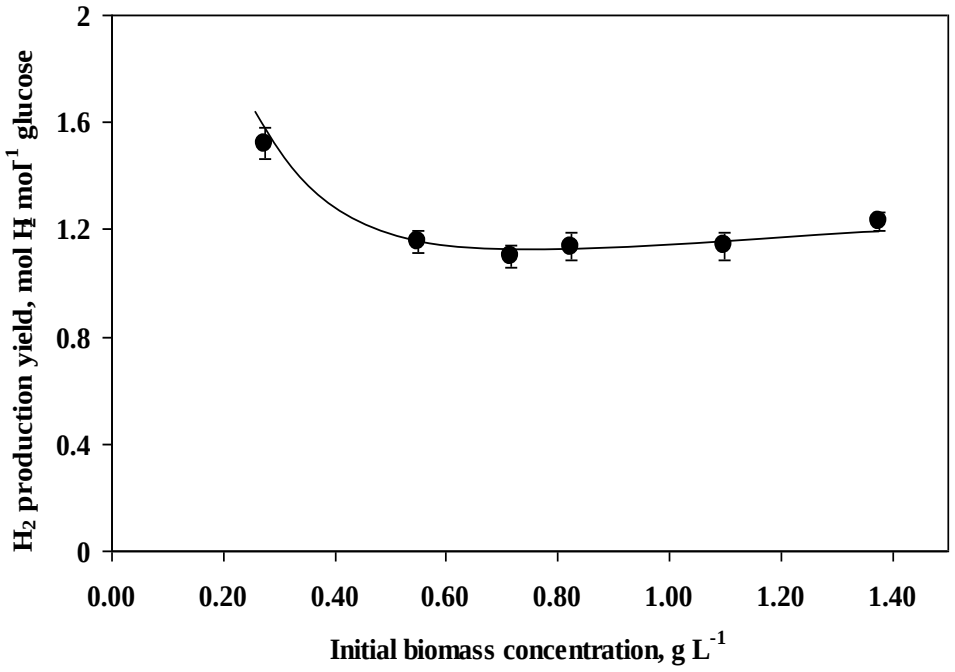

Figure 3.6 Variation of hydrogen gas yield with initial biomass concentration. S_o = 7.2 ± 0.2 g L^{-1}.

The rate and extent of hydrogen gas formation obtained in this study (dark fermentation of acid hydrolyzed wheat starch) are superior to the results of Argun et al., (2008a) study on direct dark fermentation of ground wheat where bacterial hydrolysis of boiled wheat starch and fermentation took place simultaneously. The highest cumulative hydrogen, H_2 formation rate and the yield obtained in this study were 696 ml, 30.5 ml h^{-1} and 1.46 mol H_2 mol^{-1} glucose, respectively at the initial total sugar concentration of 27.5 g L^{-1}. In the studies of Argun et al., (2008a), with 20 g L^{-1} boiled wheat starch CHF = 750 ml, R_m = 16.7 ml h^{-1} and Y = 0.8 mol H_2 mol^{-1} glucose were obtained. Duration of fermentation was also reduced from 120 h to 40 h by using sugar solution derived from acid hydrolyzed wheat starch. Lag phase durations were reduced from nearly 30 h in our previous study to nearly 4 h in this study. Partial hydrolysis of wheat starch by boiling probably formed some polysaccharides which were not readily fermentable by anaerobic bacteria. The results clearly indicated that acid hydrolysis prior to dark fermentation is more beneficial as compared to bacterial hydrolysis of boiled wheat starch during dark fermentation.

3.2 Continuous Fermentations

3.2.1 Continuous Dark Fermentation

Figure 3.7 depicts pre-steady-state variations of daily hydrogen gas production, total sugar, TVFA and NH_4–N concentrations with time for HRT = 6 h. Daily hydrogen gas production increased with time and reached a steady level of 305 ml H_2 d^{-1} after four days of operation. In parallel to hydrogen gas formation, total sugar concentration (substrate) decreased and TVFA (product) increased with time and reached steady levels after 4 days of operation. Ammonium–N concentrations were always less than 1 mg L^{-1}. Effluent total sugar content decreased from 8.6 g L^{-1} feed concentration to nearly 1.45 g L^{-1} yielding 83% total sugar fermentation at HRT = 6 h. The effluent TVFA content was nearly 3.35 g L^{-1} at steady-state. The results indicated that approximately 47% of total sugar was converted to fermentation end products as VFA. Hydrogen yield was 43 ml H_2 g^{-1}

starch at the steady-state for HRT = 6 h. ORPs varied between −200 mV and −300 mV during the operation.

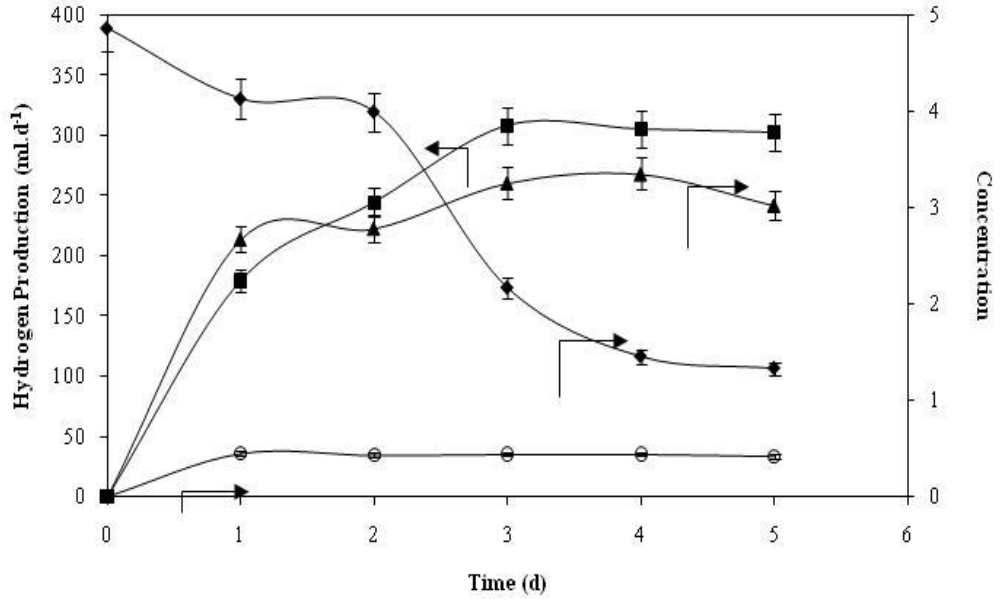

Figure 3.7 Pre-steady-state variations of (■) daily hydrogen gas production (ml d^{-1}), (♦) total sugar (g L^{-1}), (▲) total volatile fatty acids (g L^{-1}), and (○) NH_4–N (mg L^{-1}) concentrations with time for operation at HRT = 6 h.

Variation of daily hydrogen gas production with HRT is depicted in Figure 3.8. Hydrogen production rate steadily increased with decreasing HRT and reached the highest level of 305 ml H_2 d^{-1} at HRT = 6 h. Hydrogen gas production rates were above 250 ml d^{-1} for HRTs below 24 h. Further increases in HRTs above 24 h resulted in sharp decreases in hydrogen production rates yielding the lowest hydrogen production rate (40.5 ml d^{-1}) at HRT = 60 h. High total sugar loading rates at low HRTs ($L_s = Q\ S_o/V = S_o$/HRT) is probably the major reason for high daily hydrogen gas production due to high substrate availability. Composition of microbial community also changed with HRT and hydrogen gas was more effectively produced by the fast growing anaerobic bacteria at low HRTs (<24 h). ORPs decreased with decreasing HRT or increasing hydrogen gas production and varied between −200 and −300 mV.

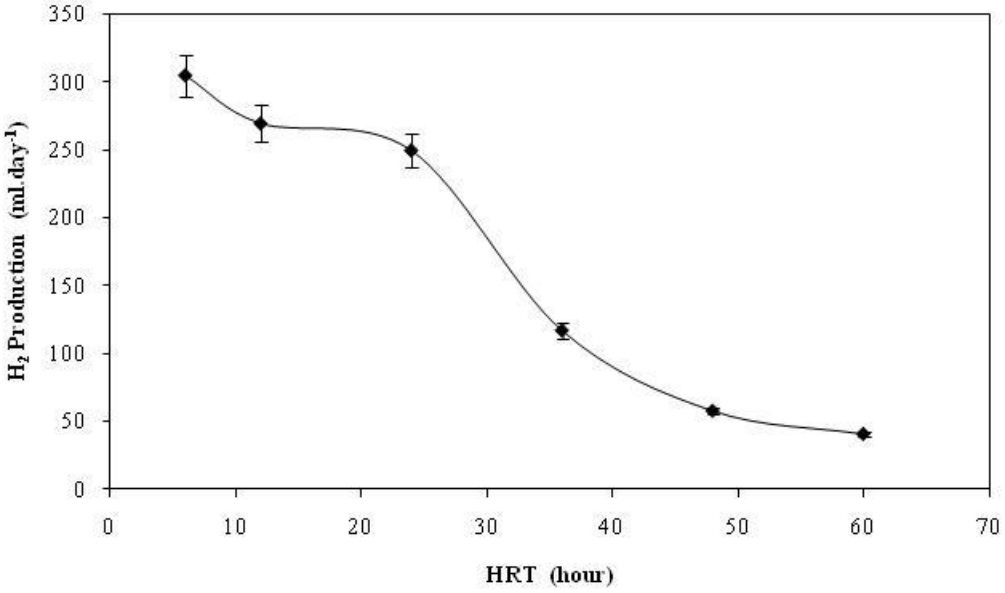

Figure 3.8 Variation of steady-state daily hydrogen production with hydraulic residence time (HRT)

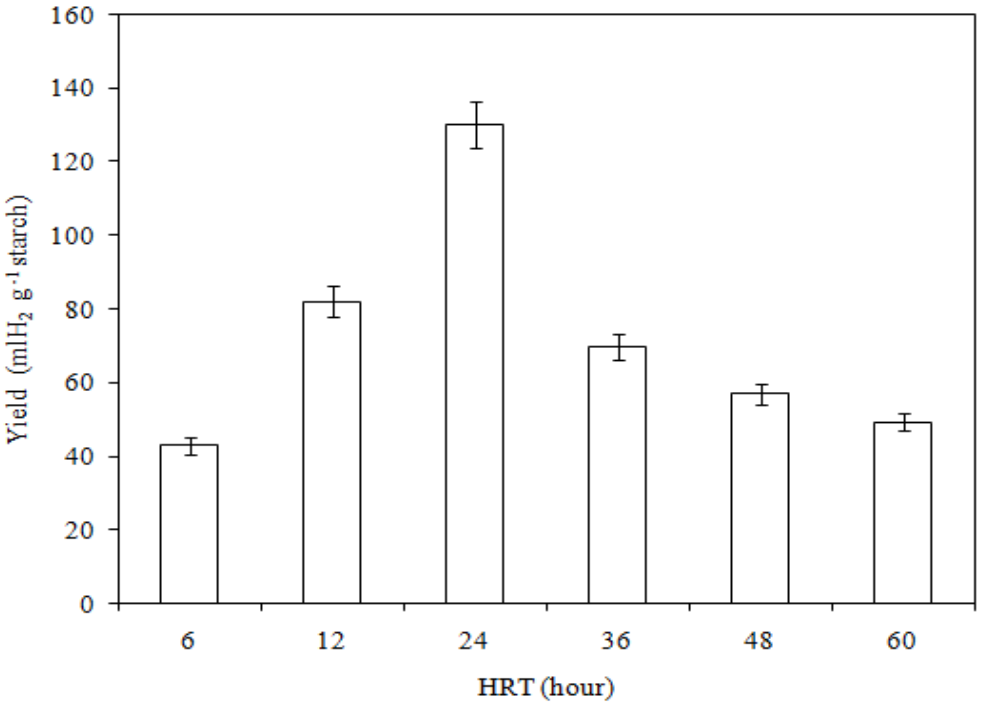

Figure 3.9 Variation of hydrogen yield with hydraulic residence time (HRT)

Variation of hydrogen yields (ml H_2 g^{-1} total sugar) with HRT is depicted in Figure 3.9. Unlike daily hydrogen gas production, hydrogen yield increased with increasing HRT and reached the highest level (130 ml H_2 g^{-1} total sugar) at HRT = 24 h. However, sharp decreases in hydrogen yields were observed at HRTs above 24 h with the lowest yield of 49 ml H_2 g^{-1} total sugar at HRT = 60 h. Hydrogen yields varied depending on the metabolic capabilities of the dominant bacterial culture present at different HRTs. In continuous culture, the growth rate is equal to the dilution rate (D = 1/HRT = Q/V) at steady-state. HRT is an important operating parameter for selection and retention of efficient hydrogen producing organisms in the system. At a certain HRT or dilution rate only the bacteria with a growth rate equal or higher than the dilution rate can survive, and the slow growing bacteria would wash out. Therefore, the composition of the bacterial population and metabolic products of dominant bacteria vary with HRT. Operation at HRT = 24 h selected the bacteria with the highest hydrogen yield. Low daily hydrogen productions (V_{H2}) and total sugar loading rates (L_s) resulted in low hydrogen yields at high HRTs (>24 h). At low HRT operations (<24 h), despite high daily hydrogen productions, hydrogen gas yields were low due to unfavorable metabolic products of the dominant bacteria. The optimum HRT yielding the highest hydrogen yield was 24 h.

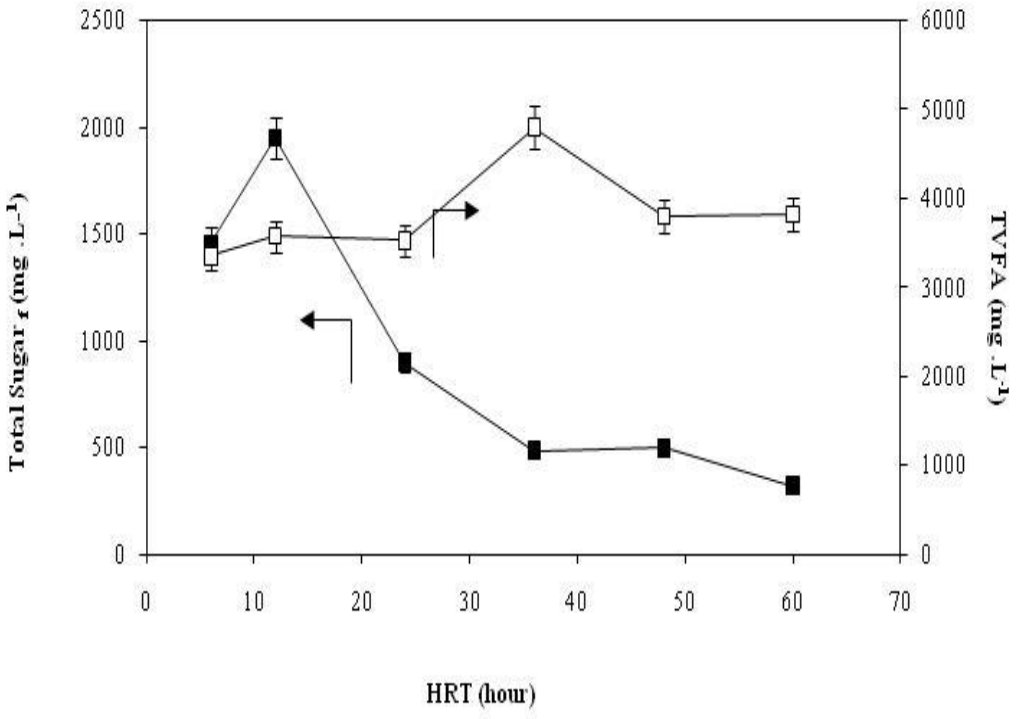

Figure 3.10 Variation of steady-state effluent total sugar and TVFA concentrations with hydraulic residence time (HRT)

Figure 3.10 depicts variation of steady-state total sugar (TS) and total volatile fatty acid (TVFA) concentrations with HRT. The feed total sugar concentration was constant at 9 ± 0.5 g L^{-1} constituting mainly glucose (95%) and small amount of starch (5%). Total sugar concentration decreased steadily with increasing HRT indicating effective fermentation of carbohydrates at high HRTs. The effluent total sugar was less than 0.5 g L^{-1} for HRTs higher than 36 h indicating nearly 95% sugar consumption. Effluent TVFA concentrations varied between 3350 (HRT = 6 h) and 4800 mg L^{-1} (HRT = 36 h). The average effluent TVFA was around 3500–4000 mg L^{-1} indicating nearly 45% conversion of total sugars to TVFAs by dark fermentation. Despite relatively low extent of total sugar fermentation (83%), TVFA formation and daily hydrogen production rate was high at HRT = 6 h due to high total sugar loading rate (nearly 34 g TS L^{-1} d^{-1}). Apparently, the fast growing bacteria at low HRTs converted sugar compounds to VFAs and hydrogen gas more effectively using proper metabolic pathways.

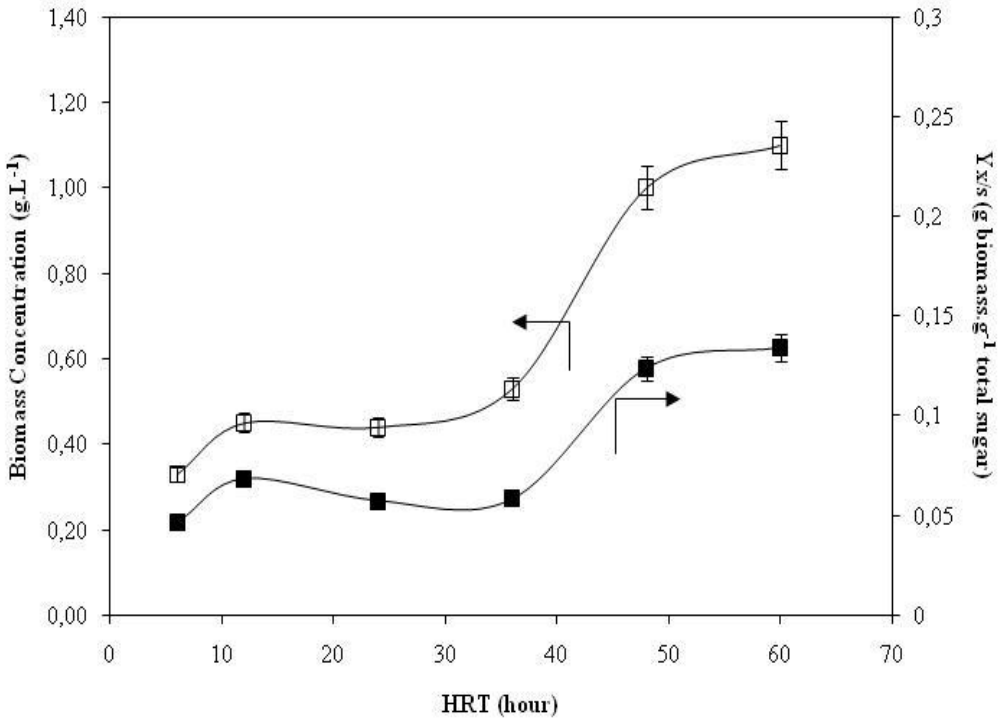

Figure 3.11 Variation of steady-state biomass concentration and biomass yield with hydraulic residence time (HRT).

Variations of steady-state biomass concentrations (X, g L^{-1}) and the growth yield coefficient ($Y_{x/s}$, gX g^{-1} total sugar) with hydraulic residence time are depicted in Figure 3.11. Since, the feed total sugar was fermented more effectively at high HRTs, the steady-state biomass concentration ($X = Y_{x/s}$ ($S_o - S$)), increased with HRT as expected. The increase in biomass concentration was rather slow at low HRTs and became larger at high HRT operations. Steady-state biomass concentration increased from 0.35 g L^{-1} to 1.15 g L^{-1} when HRT was increased from 6 h to 60 h. The growth yield coefficient ($Y_{x/s} = \Delta X/\Delta S$) also increased from nearly 0.040 gX g^{-1}S to 0.12 gX g^{-1}S when HRT was increased from 6 to 60 h due to high biomass concentrations at high HRTs. Apparently, most of the carbohydrates were used for growth rather than product formation at high HRTs.

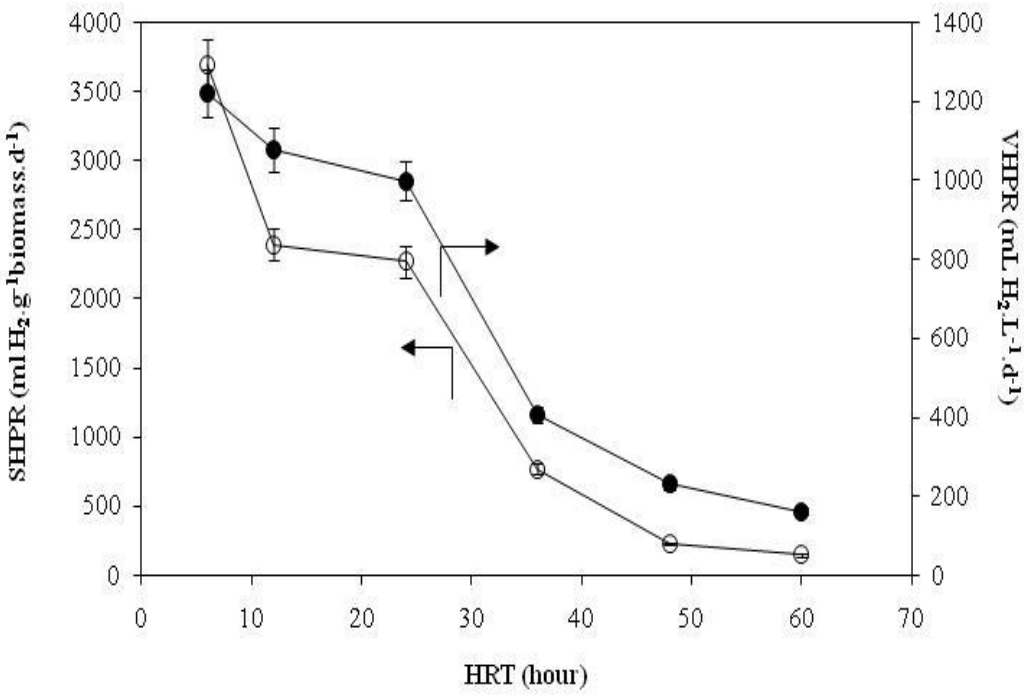

Figure 3.12 Variation of volumetric (VHPR) and specific hydrogen gas production rates (SHPR) with hydraulic residence time (HRT).

Steady-state volumetric (R_v, ml H_2 L^{-1} d^{-1}) and specific (R_x, ml H_2 g^{-1} biomass d^{-1}) rates of hydrogen gas formation were calculated by using the equations 3.5 and 3.6 and were plotted against HRT in Figure 3.12. Since the steady-state daily hydrogen formation reached the highest level (305 ml d^{-1}) at HRT = 6 h, volumetric rate of hydrogen production was also the highest (1220 ml H_2 L^{-1} d^{-1}) at HRT = 6 h. Specific rate of hydrogen formation also reached the highest level (3485 ml H_2 g^{-1} biomass d^{-1}) at HRT of 6 h. Further increases in HRT resulted in decreases in volumetric and specific rates of hydrogen formation due to low total sugar loading rates at high HRTs.

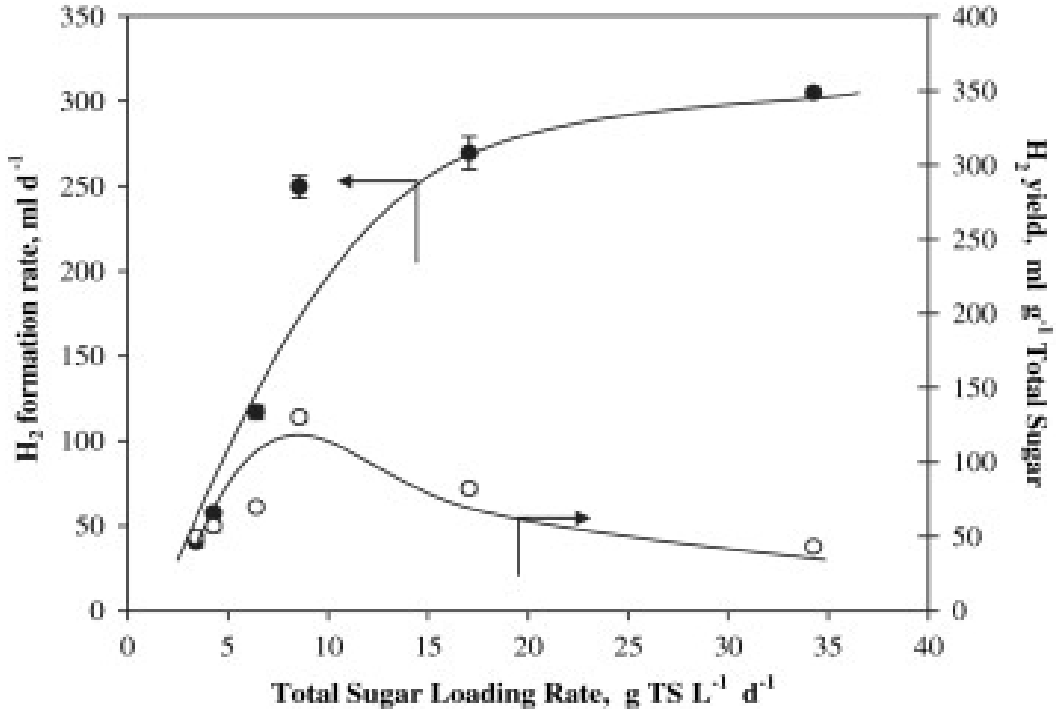

Figure 3.13 Variation of hydrogen gas yield and formation rate with total sugar loading rate.

Total sugar (substrate) loading rate (L_s, g TS L^{-1} d^{-1}) is an important factor affecting the rate of hydrogen gas formation. Hydrogen gas formation rate (ml H_2 d^{-1}) increased with total sugar loading rate as depicted in Figure 3.13 due to high substrate availability at high sugar loading rates. The highest daily hydrogen production (305 ml d^{-1}) was observed at total sugar loading rate of 34 g TS L^{-1} d^{-1} at the lowest was at HRT of 6 h. However, hydrogen yield reached the highest level at a total sugar loading rate of 8.5 g TS L^{-1} d^{-1} at HRT of 24 h. The hydrogen yield is closely related to the type of dominant bacteria and their metabolic products rather than total sugar loading rate. For this reason the optimum sugar loading rate maximizing the yield are different from that of the maximum hydrogen formation rate.

Table 3.5 VFA composition of dark fermentation effluent at different HRTs.

HRT (h)	**6**	**12**	**24**	**36**	**48**	**60**
Lactic (mg L^{-1})	775	84	111	365	102	147
Acetic (mg L^{-1})	2334	2763	1284	1110	582	489
Butyric (mg L^{-1})	165	436	2040	2496	2442	2232
Propionic (mg L^{-1})	0	0	0	51	93	311
Total VFA	3274	3283	3435	4022	3219	3179
TVFA by anal. kits	3350	3575	3525	4800	3800	3825
Acetate/butyrate(M/M)	20,4	9,3	0,92	0,65	0,35	0,32

Table 3.5 summarizes effluent VFA concentrations determined by HPLC analyses at different HRTs. Dark fermentation effluent contained mainly lactic, acetic, butyric and propionic acids. TVFA concentrations determined by analytical kits were slightly higher than the sum of individual VFAs due to presence of some unidentified peaks in HPLC analyses. Composition of VFAs varied with operating HRT due to variations in composition of anaerobic bacterial consortium. In acetic and butyric acid fermentations, 2 mol H_2/mole acetic acid and 1 mol H_2/mole butyric acid are produced, respectively. Propionic acid fermentation consumes 1 mol of H_2/mole propionic acid and no hydrogen is produced in lactic acid fermentation. In terms of hydrogen gas production, acetic and butyric acids are preferred end products in dark fermentation of carbohydrates. Acetic acid formation decreased with increasing HRT above 6 h resulting in lower hydrogen formation at high HRTs. Unlike acetic acid, butyric acid formation increased with increasing HRTs up to 60 h. Since, butyric acid formation is accompanied with lower hydrogen yield as compared to acetic acid, relatively high butyric acid formation at high HRTs resulted in low hydrogen yields. Lactic and propionic acid formations were much lower as compared to acetic and butyric acids. Therefore, hydrogen formation was mainly determined by the acetic/butyric acid ratio in the effluent. High acetic/butyric acid molar ratio yielded

high hydrogen formations at low HRTs. In fact, the molar ratio of acetate/butyrate decreased from 20.4 to 0.32 when HRT was increased from 6 h to 60 h.

Table 3.6 Comparison of hydrogen yields of continuous dark fermentation studies.

Seed Culture	**Substrate**	**H_2 Yield (ml $H_2 g^{-1}$starch)**	**HRT**	**Ref**
Sewage sludge	Wheat feed (20 g L^{-1})	55.09	15 h	Hawkes et al. (2007)
Anaerobic digester sludge	Corn starch (20 g $CODL^{-1}$)	139.63	12 h	Arooj et al. (2008)
Clostridium butyricum CGS2	Hydrolyzed starch (26 g L^{-1})	227.75	2 h	Chen et al. (2008)
Anaerobic sludge	Starch (12 g L^{-1})	151.83	3 h	Li et al. (2010)
Granular anaerobic sludge	Starch (20 g $CODL^{-1}$)	124.23	0.5 h	Cheng et al. (2008)
Sludge from paper mill wastewater treatment plant	Starch (20 g $CODL^{-1}$)	227	4 h	Lin et al. (2008)
Heat treated anaerobic sludge	Acid hydrolysed wheat starch (10 g L−1)	130	24 h	This study

A number of continuous dark fermentation studies were reported in literature for bio-hydrogen production from different substrates (Hawkes et al., (2007); Chen et al., (2008); Arooj et al., (2008); Li et al., (2010); Cheng et al., (2008); Lin et al., (2008)). The results of this study are compared with other continuous dark fermentation studies in Table 3.6. The highest hydrogen yield obtained in this study (130 ml g^{-1} total sugar) is higher than some of the reported studies Hawkes et al., (2007), and Cheng et al., (2008) is comparable to some others Arooj et al., (2008) and Li et al., (2010). However, higher hydrogen yields were reported in some studies as compared to this study Chen et al., (2008), and Lin et al., (2008). The reason for differences is the fact that different substrates, bacterial cultures and conditions were used in different studies. Variation in composition of bacterial cultures and metabolic

products with operating HRT and the type of substrate cause variations in composition of VFAs and therefore, hydrogen yields and formation rates. Further improvements in hydrogen yields can be obtained by directing the metabolic pathways of the bacteria towards mainly acetic and butyric acid formations by using pure or defined mixed cultures under optimum environmental conditions.

3.2.2 Continuous Photo-Fermentation

3.2.2.1 Pre-steady-state hydrogen gas production

Continuous photo-fermentation studies were carried out at five different HRTs between 1 and 10 days. Periodic feeding and removal rates were varied between 250 ml d^{-1} and 25 ml d^{-1} to obtain different HRTs while the fermentation volume was constant at 0.25 L. The system was operated until the steady-state was reached for every operation at different HRTs. Figure 3.14 depicts time course of pre-steady-state variations of daily H_2 gas production, TVFA and NH_4-N concentrations for the last eight days of continuous operation at HRT = 8 days. Daily H_2 gas production and TVFA concentration increased with operation time and reached steady levels of 70 ml H_2 d^{-1} and 0.72 g TVFA L^{-1}, respectively after 68 days. Feed flow rate and feed TVFA concentrations at HRT = 8 days were 31 ml d^{-1} and 2.62 g L^{-1}, respectively yielding TVFA loading rate of 0.078 g TVFA d^{-1}. Effluent TVFA concentration was 0.72 g L^{-1} yielding TVFA fermentation rate of 0.059 g TVFA d^{-1} and hydrogen gas yield of 1186 ml H_2 g^{-1} TVFA at the steady-state of operation at HRT = 8 days. This yield is one of the highest photo-fermentative hydrogen yields reported in literature.

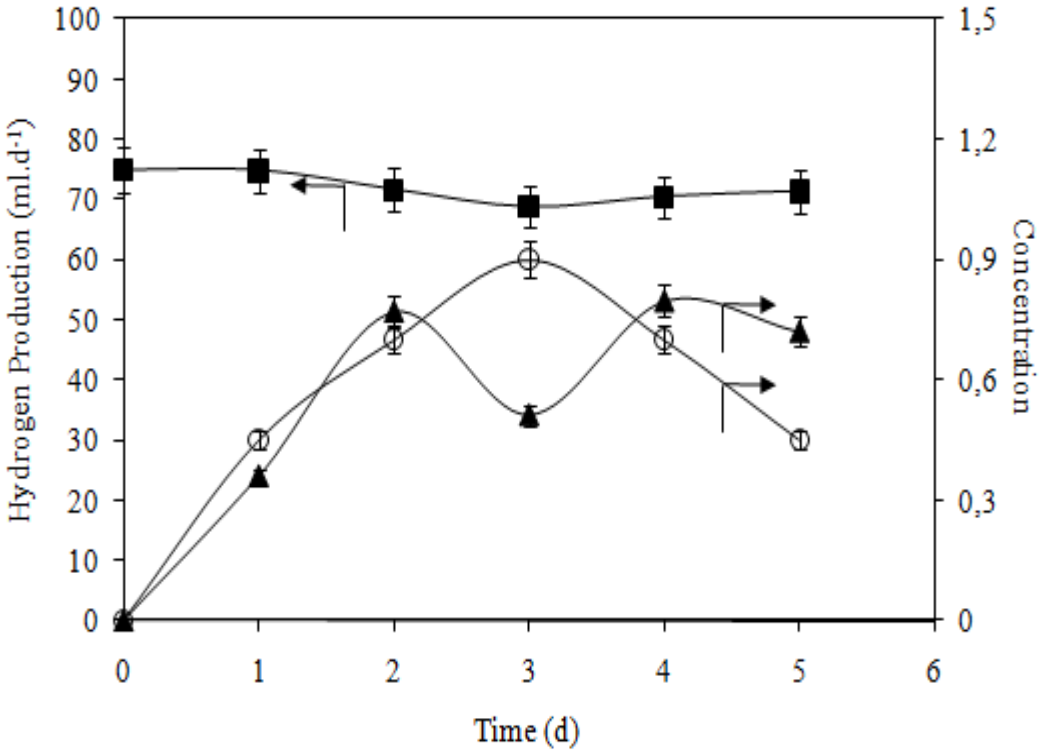

Figure 3.14 Pre-steady state variations of (■) daily hydrogen gas production (ml d^{-1}), (▲) total volatile fatty acids (g L^{-1}) and (○) NH_4-N concentrations (mg L^{-1}) with time for operation at HRT= 8 days

3.2.2.2 Steady-state H_2 gas and biomass production rates and the yields

Daily H_2 gas production rates were measured during the whole operation period for every experimental bottle operating at different HRTs. Steady-state daily H_2 gas production rates were plotted against HRT in Figure 3.15. Hydrogen gas production rate increased with increasing HRT between 24 h (1 d) and 96 h (4 d) and reached the highest level (85 ml d^{-1}) at HRT = 96 h (4 d). Further increases in HRT resulted in decreases in daily H_2 gas formation rate resulting in 30 ml d^{-1} at HRT = 240 h (10 d). This finding is in agreement in continuous culture theory where product and biomass formation rates reach the highest level at the optimum HRT or dilution rate ($D = Q/V = 1/\text{HRT}$). In this study, the optimum HRT yielding the highest rate of product formation was 96 h (4 days).

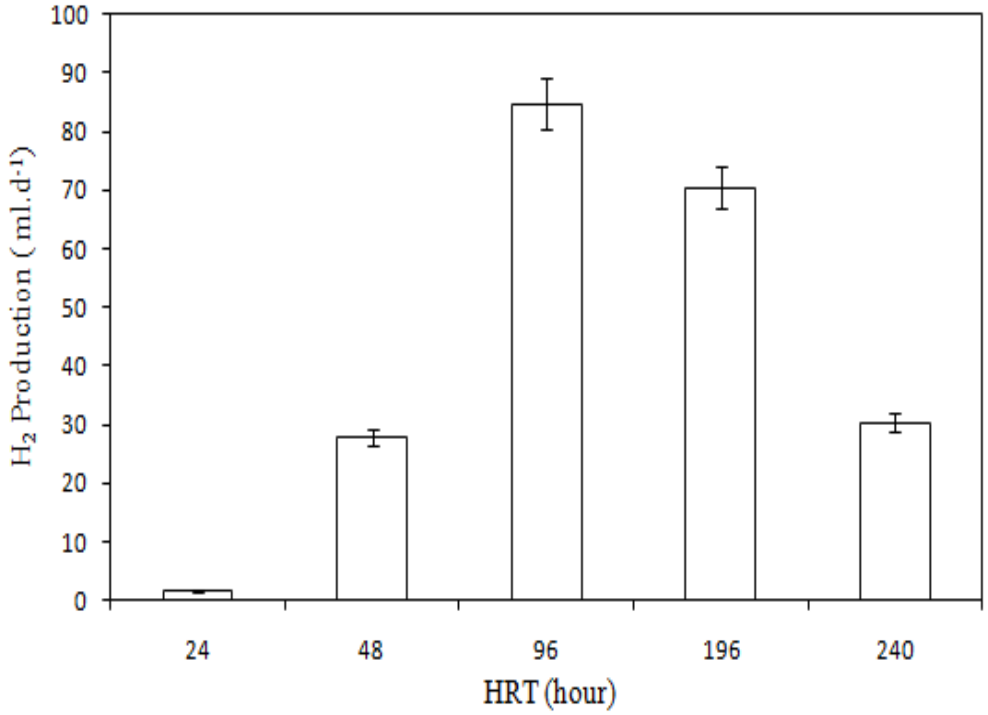

Figure 3.15 Variation of steady-state daily hydrogen gas production with hydraulic residence time (HRT)

The rate and the yield of product formation are the two most important parameters to be maximized in continuous operation. Figure 3.16 depicts variation of steady-state H_2 gas yield with operating HRT. Similar to hydrogen formation rate (HFR), hydrogen yield (HY) also increased with increasing HRT and reached the highest level (1200 ml H_2 g^{-1} TVFA) at HRT = 196 h (8.1 d). Further increases in HRT resulted in decreases in HFR yielding 840 ml g^{-1} TVFA at HRT = 240 h (10 d). Hydrogen yield at HRT = 96 h (4 d) was 1035 ml H_2 g^{-1} TVFA which was only 14% lower than that obtained at HRT = 196 h. The optimum HRT maximizing the hydrogen yield was different from that of hydrogen formation rate.

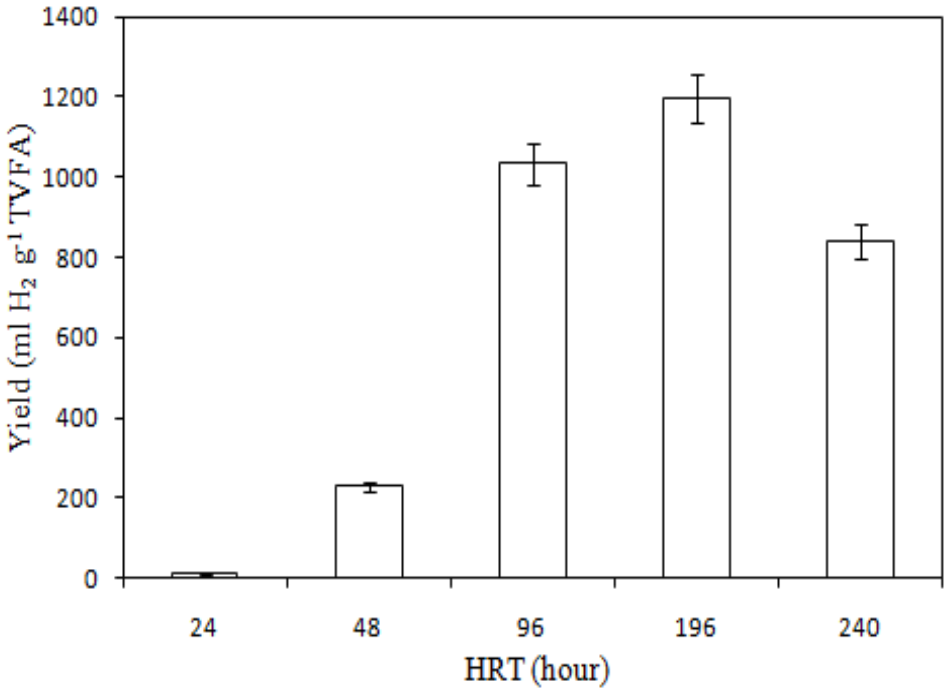

Figure 3.16 Variation of hydrogen gas yield with hydraulic residence time (HRT).

Variations of steady-state biomass (*Rhodobacter* sp) concentrations and biomass growth yields are depicted in Figure 3.17. Biomass (dry cell weight) concentrations at steady-states increased from 0.3 g L^{-1} to 3.64 g L^{-1} when HRT was increased from 24 h (1 d) to 240 h (10 d). Increase in biomass concentration for HRTs between 1 and 8 days was almost linear. The growth yield coefficient ($Y_{x/s}$, g biomass g^{-1} TVFA) also increased with increasing HRT indicating utilization of VFAs for growth rather than product (H_2 gas) formation at high HRTs. Therefore, high biomass yield coefficients at high HRTs resulted in low hydrogen yield coefficients.

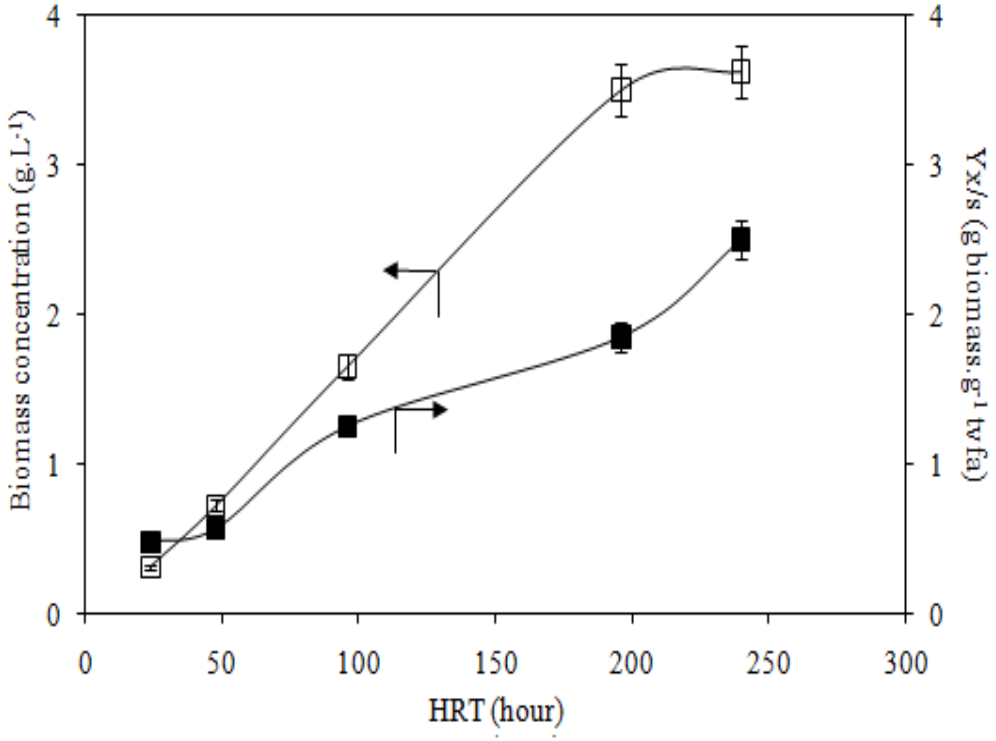

Figure 3.17 Variation of steady-state biomass concentration and biomass yield with hydraulic residence time (HRT)

3.2.2.3 Volumetric and specific hydrogen gas formation rates

Volumetric (VHPR, ml H_2 L^{-1} d^{-1}) and specific (SHPR, ml H_2 g^{-1} biomass d^{-1}) hydrogen gas production rates are important parameters used in process design as explained in section 3.2. Figure 3.18 depicts variation of VHPR and SHPRs with HRT. Both rates increased with increasing HRT up to HRT = 96 h (4 d) and reached the highest level. Further increases in HRT caused decreases in VHPR and SHPR. Therefore, just like daily hydrogen production rate, the optimum HRT maximizing VHPR (340 ml L^{-1} d^{-1}) and SHPR (205 ml H_2 g^{-1} d^{-1}) was 4 days (96 h). High biomass concentrations at HRTs above 4 days resulted in low SHPRs.

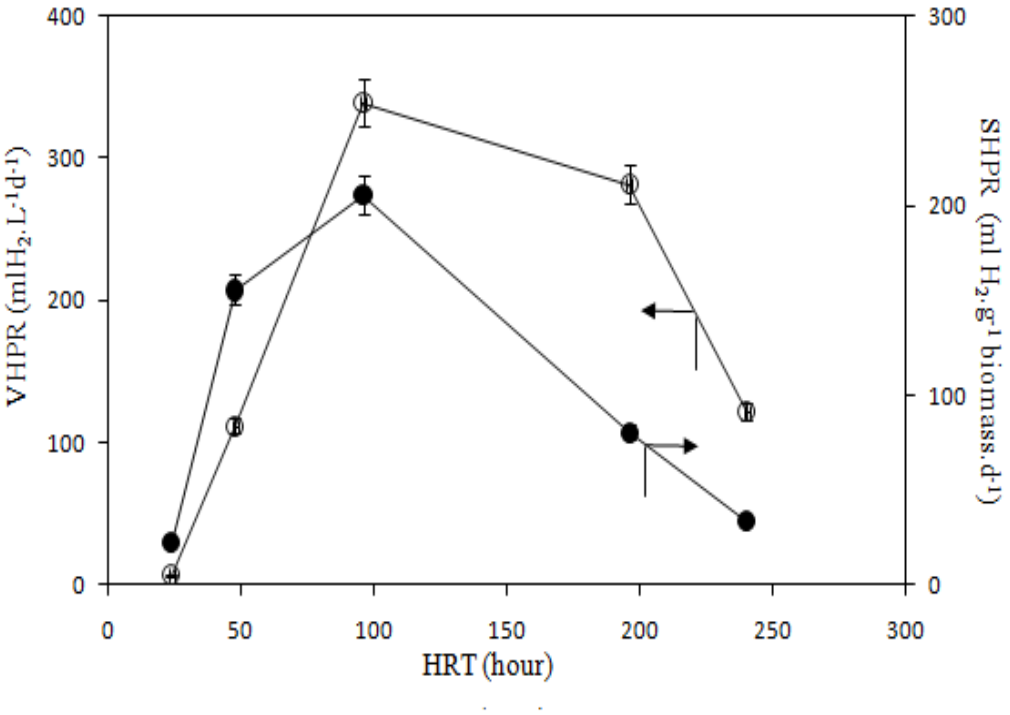

Figure 3.18 Variation of volumetric (VHPR) and specific hydrogen gas production rates (SHPR) with hydraulic residence time (HRT).

Substrate (TVFA) loading rate (Ls , g TVFA L^{-1} d^{-1}) is an important factor determining hydrogen gas formation rate and the yield since product formation is closely related to the substrate availability. Substrate loading rates varied between 2.02 and 0.23 g TVFA L^{-1} d^{-1} when HRT was varied between 1 and 10 days. Figure 3.19 depicts variation of hydrogen gas formation rate and the yield with the substrate (TVFA) loading rate. The highest hydrogen gas formation rate was obtained at 0.5 g TVFA L^{-1} d^{-1} and the highest yield was at the loading rate of 0.32 g TVFA L^{-1} d^{-1} corresponding HRTs of 4 and 8 days, respectively. Since the operation at HRT = 4 days (96 h) and substrate loading rate of 0.5 g TVFA L^{-1} d^{-1} yielded the highest H_2 gas formation rate (85 ml H_2 d^{-1}) and a hydrogen yield (1035 ml H_2 g^{-1} TVFA) very close to the highest level, operation at HRT = 96 h (4 d) is recommended.

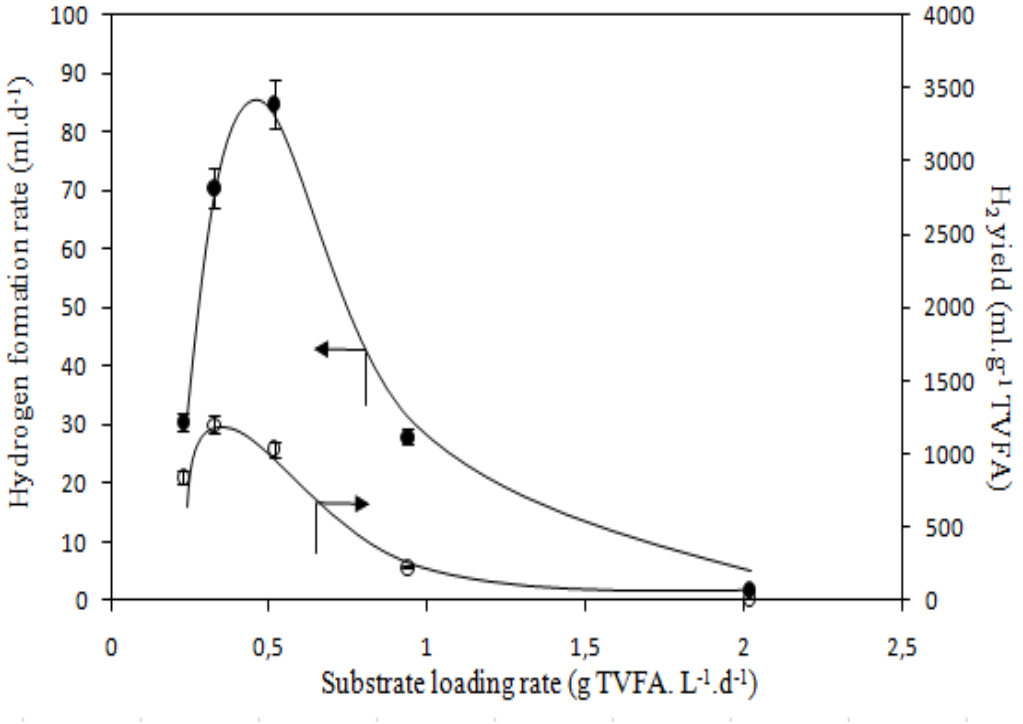

Figure 3.19 Variation of hydrogen gas yield and formation rate with the substrate (TVFA) loading rate

The results of this study are compared with the literature studies on photo-fermentative hydrogen production from pure VFAs in Table 3.7. Most of the literature studies on photo-fermentation are batch fermentations (Barbosa et al., (2001); Koku et al., (2003); Oh et al., (2004); Shi & Yu, (2004); Shi & Yu, (2005); Argun et al., (2008b); Kapdan et al., (2009); Argun & Kargi, (2010b)). There is limited number of continuous fermentation studies on bio-hydrogen production from VFAs (Ozmihci & Kargi, (2010b); Fascetti et al., (1998); Najafpour et al., (2003); Jeong et al., (2007); Fascetti & Todini, (1995)). The rate of hydrogen gas formation obtained in this study is comparable to the reported studies (Fascetti & Todini, (1995); Chen et al., (2007); Basak & Das, (2009)). As compared to study of Ozmihci & Kargi, (2010b) on photo-fermentative H_2 gas production from dark fermentation effluent of ground wheat (no acid hydrolysis), this study resulted in considerably higher hydrogen formation rates and the yields (Table 4.7). The highest hydrogen yield (1200 ml H_2 g^{-1} TVFA) obtained in this study is one of the highest reported in literature. The results of this study indicated that acid hydrolysis of wheat starch

before dark fermentation yields more favourable VFA composition in dark fermentation effluent to be used in photo-fermentation. Utilization of pure VFAs for photo-fermentative H_2 gas production may not be economically feasible due to high cost of pure raw materials. However, dark fermentation effluent of acid hydrolyzed wheat starch contains a mixture of VFAs and can be considered as wastewater. The performance of photo-fermentation largely depends on the composition of VFAs in dark fermentation effluent and the type of bacteria used. Apparently, VFA composition of dark fermentation effluent of acid hydrolyzed wheat starch was more favourable for H_2 gas production by photo-fermentation.

Table 3 Comparison of photo-fermentative H_2 gas production from volatile fatty acids.

Inoculum culture	**C-source**	**N-source**	**Light source**	**Light intensity**	**SHPR**	**HPR**	**Yield**	**Ref.**
Rhodopseudomonas palustris AHWS3-5	Butyric acid (1.83 g L^{-1})	Glutamic acid 0.607 g L^{-1}	Tungsten	10 klux	–	24.9 ml H_2 L^{-1} h^{-1}	5.74 mol/mol HBu = 1460 ml g^{-1} HBu	Chen et al. (2007)
Rhodobacter sphaeroides OU 001	dl-malic acid (2.01 g L^{-1})	Glutamate 0.3 g L^{-1}	Tungsten	15 ± 1.1 W m^{-2}	5.42 ml H_2 g^{-1} h^{-1}	6.5 ml H_2 L^{-1} h^{-1}	4.5 mol/mol HMal = 751 ml g^{-1} HMal	Basak & Das (2009)
Rhodobacter sphaeroides RV NIBH-8703	Lactic acid (100 mM = 9 g L^{-1})	Yeast extract 0.5 g L^{-1}	Tungsten	10 klux	75 ml H_2 g^{-1} h^{-1}	66.6 ml H_2 L^{-1} h^{-1}	4.8 mol/mol HLa = 1194 ml g^{-1} Hlac	Fascetti & Todini (1995)
Rhodobacter sphaeroides NRRL B-1727	Dark ferm effluent of acid hydrolyzed wheat starch	None	Halogen lamp	5 klux	205 ml g^{-1} d^{-1}	340 ml L^{-1} d^{-1} (85 ml d^{-1})	1200 ml g^{-1} TVFA	This study
Rhodobacter sphaeroides NRRL B-1727	Dark ferm effluent of ground wheat starch	None	Halogen lamp	5 klux	77 ml g^{-1} d^{-1}	27 ml L^{-1} d^{-1} (55 ml d^{-1})	185 ml g^{-1} TVFA	Ozmihci & Kargi (2010b)

3.2.3 Continuous Combined Fermentation

Figure 3.20 depicts variation of daily hydrogen gas production with HRT. Hydrogen production rate was 25 ml d^{-1} at the steady-state for HRT = 1 day which decreased to 6.3 ml d-1 at HRT = 2 days. At low HRTs only dark fermentation was functional yielding relatively high H_2 formation rate at HRT = 1 day. Further increases in HRT yielded higher H_2 formation rates probably due to contribution of photo-fermentation. The highest hydrogen formation rate (41 ml d^{-1}) was obtained at HRT = 8 days due to extra H_2 gas formation by photo-fermentation of VFAs in addition to dark fermentation of carbohydrates.

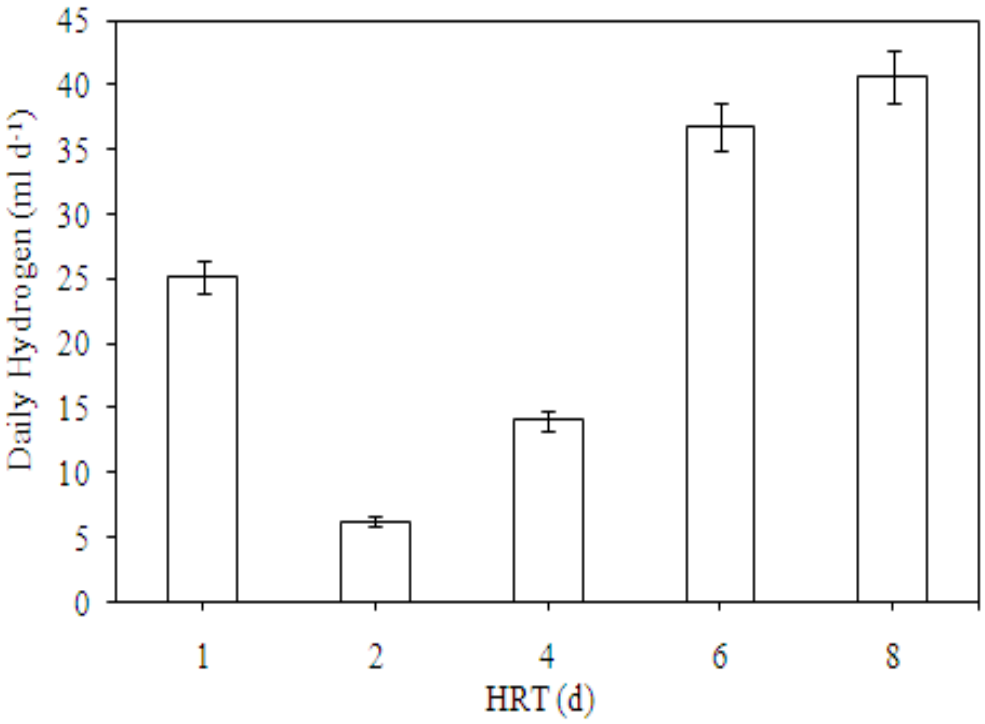

Figure 3.20 Variation of steady-state daily hydrogen gas production with hydraulic residence time

Variations of steady-state total sugar and TVFA concentrations with HRT are depicted in Figure 3.21. Feed total sugar concentration was around 5.1 ± 0.1 g L^{-1}, while the effluent total sugar varied depending on the HRT. The highest effluent total sugar (3.4 g L^{-1}) was obtained with HRT = 1 d and the lowest (0.59 g L^{-1}) with the HRT of 8 d indicating effective fermentation of sugar at high HRTs for H_2 gas production. TVFAs were produced as the intermediate product from dark fermentation of carbohydrates which were fermented by the photo-fermentation bacteria to H_2 and CO_2. The initial TVFA concentrations were around 0.3 ± 0.05 g L^{-1} carried with the inoculum. The effluent TVFA concentrations varied depending on the HRT. The effluent TVFA at HRT = 1 day was 0.295 g L^{-1} due to low extent of dark fermentation at low HRT. The effluent TVFA for HRTs 2 to 6 days were around 3.5 ± 0.3 g L^{-1} indicating effective VFA and H_2 gas formation by dark fermentation. Photo-fermentative VFA fermentation took place at HRT = 8 days yielding low effluent TVFA (2.0 g L^{-1}) and high daily H_2 gas production. Further increases in HRT might have resulted in lower effluent TVFAs and higher volumes of H_2 gas formation.

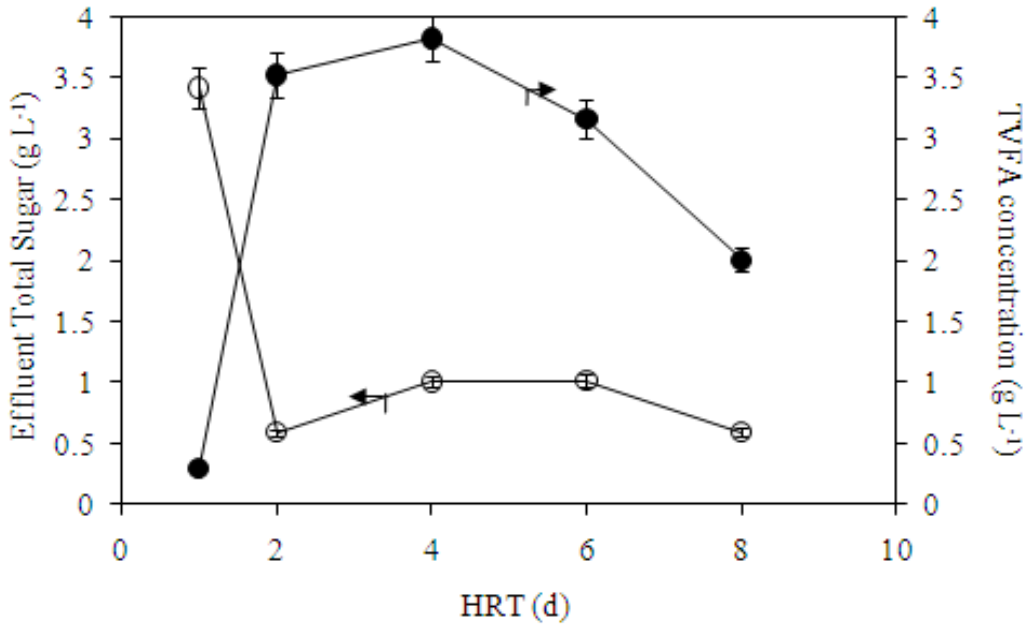

Figure 3.21 Variation of effluent total sugar and TVFA concentrations with HRT at steady-state. (o) Effluent total sugar (•) Effluent TVFA

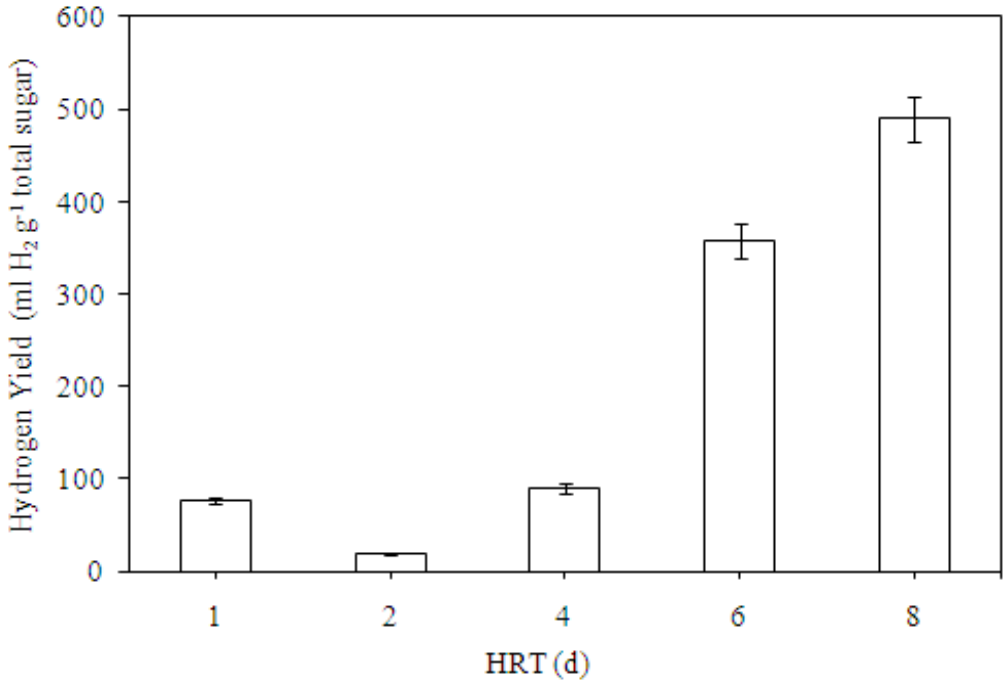

Figure 3.22 Variation of hydrogen gas yield with hydraulic residence time (HRT)

Figure 3.22 depicts variation of hydrogen yields (ml H_2 g^{-1} total sugar) with HRT. Hydrogen yield steadily increased with increasing HRT (except HRT = 2 d) and reached the highest level (470 ml H_2 g^{-1} total sugar) at HRT = 8 d. The lowest yield (19 ml H_2 g^{-1} total sugar) was obtained at HRT = 2 d due to H_2 formation by dark fermentation alone. At high HRTs above 4 days, H_2 production took place by both the dark and photo fermentations yielding higher hydrogen yields as compared to the yields of low HRTs. Operation at HRT = 8 days or longer is recommended for high H_2 yields in combined continuous fermentation.

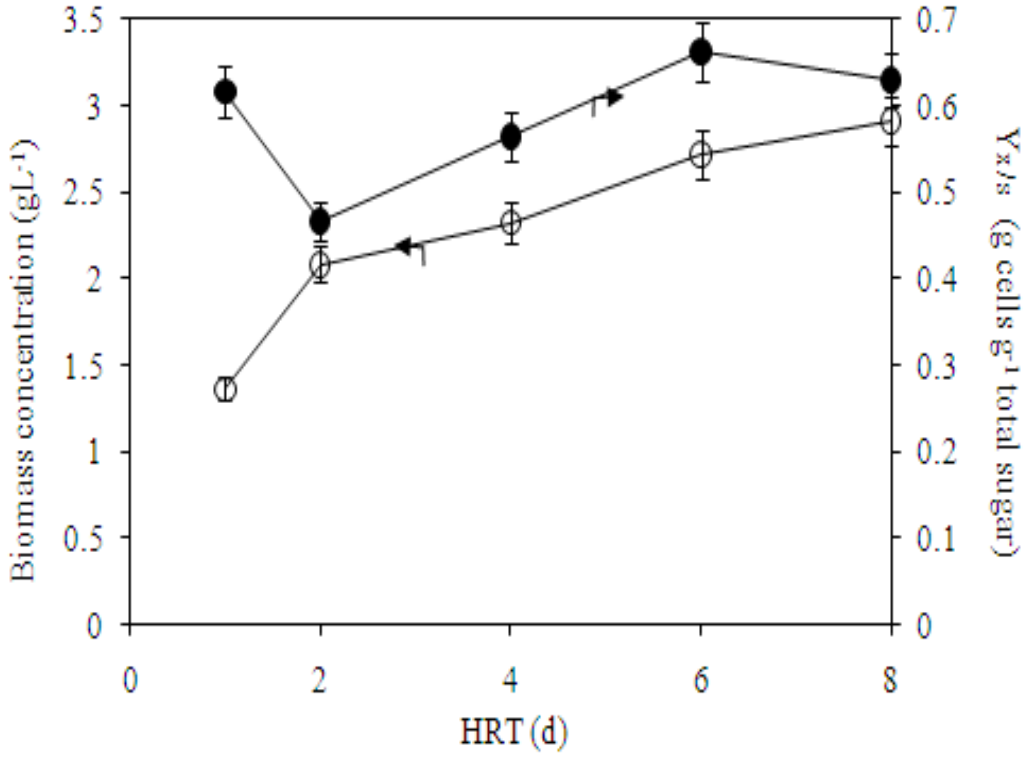

Figure 3.23 Variation of steady-state biomass concentration and biomass yield with hydraulic residence time (HRT) o Biomass concentration • Biomass yield

Variations of steady-state biomass concentrations (X, gL^{-1}) and the growth yield coefficient ($Y_{x/s}$, gX g^{-1} total sugar) with hydraulic residence time are depicted in Fig 3.23. Since, the feed total sugar was fermented more effectively at high HRTs, the steady-state biomass concentration, increased with HRT as expected. Biomass concentration at HRT = 2 d was low due to lower extent of total sugar utilization. Steady-state biomass concentration increased from 1.36 g L^{-1} to 2.91 g L^{-1} when HRT was increased from 1 to 8 days. The growth yield coefficient ($Y_{x/s}$) increased slightly with HRT. $Y_{x/s}$ was around 0.60 ± 0.6 gX g^{-1} total sugar at all operating HRTs. The lowest biomass yield was 0.47 gX g^{-1} total sugar at HRT = 2 d and the highest was 0.66 gX g^{-1} total sugar at HRT = 6 days. Slightly higher biomass concentrations at high HRTs (6-8 days) resulted higher volumes of H_2 gas formation.

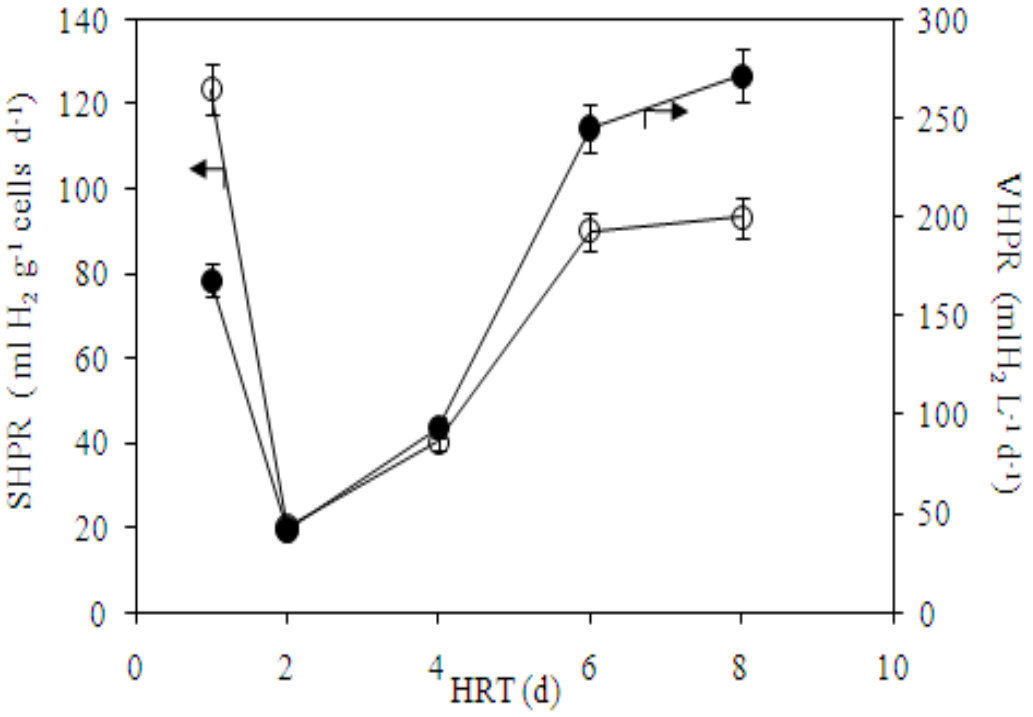

Figure 3.24 Variation of volumetric (VHPR) and specific hydrogen gas production rates (SHPR) with hydraulic residence time (HRT) • Volumetric hydrogen production rate (VHPR) o Specific hydrogen production rate (SHPR)

Figure 3.24 depicts variation of volumetric (VHPR) and specific hydrogen production rates (SHPR) with HRT. The highest VHPR (271.5 ml H_2 L^{-1} d^{-1}) was obtained at HRT = 8 days due to contribution of photo-fermentative H_2 gas production in addition to dark fermentation at high HRTs. The lowest VHPR was obtained at HRT = 2 days where daily H_2 production was also the lowest due to low extent of sugar fermentation. Specific hydrogen production rate (SHPR) showed the same trend as that of the VHPR and reached the highest level (93.5 ml H_2 g^{-1} biomass d^{-1}) at HRT of 8 days. High rates of H_2 production at high HRTs were due to combined dark and photo-fermentative H_2 gas production. At low HRTs (1-2 days) total sugar was fermented by both dark and photo-fermentation bacteria producing VFAs, H_2 and CO_2. Produced VFAs were not fermented to H_2 and CO_2 at low HRTs since photo-fermentative bacteria requires a long lag period before switching the metabolism from sugar to VFA fermentation. At high HRTs (6-8 days), VFAs were

also fermented by photo-fermentative bacteria (*Rhodobacter sp*) for production of H_2 gas.

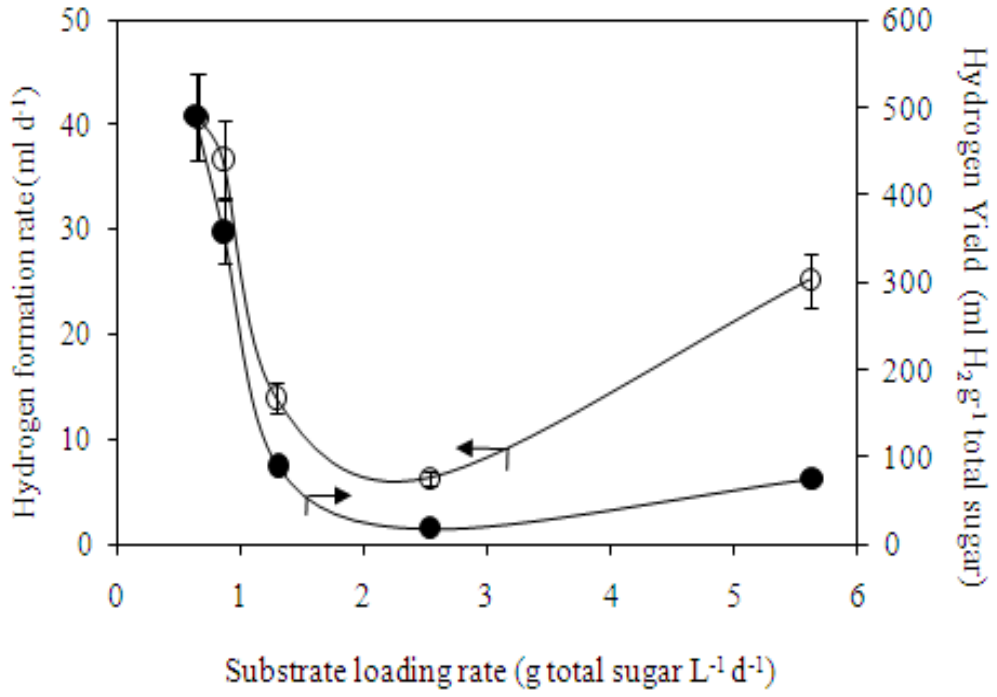

Figure 3.25 Variation of hydrogen gas yield and formation rate with the substrate (total sugar) loading rate . o Hydrogen formation rate • Hydrogen yield

Total sugar (substrate) loading rate (g TS L^{-1} d^{-1}) is the most important parameter affecting the yield and the rate of hydrogen gas formation. Variations of hydrogen yield and formation rate with HRT showed a similar trend (Fig 3.25). The highest H_2 yield and formation rate were obtained at the lowest substrate loading rate corresponding to the highest HRT of 8 d. The yield and formation rate decreased with increasing substrate loading rate (or decreasing HRT) and reached the lowest level at the loading rate of 2.5 g total sugar L^{-1} d^{-1}. Further increases in substrate loading rate resulted in slight increases in hydrogen yield and formation rate. At low substrate loading rates or high HRTs (6-8 d), H_2 was produced by both the dark and photo-fermentation with a high rate. At high substrate loading rates or low HRTs, H_2 was produced by fast growing dark fermentation bacteria yielding relatively high H_2

formation rate. At the intermediate loading rates or HRTs (3-4 d) H_2 formation was low due to ineffective dark and photo-fermentation.

Limited number of combined fermentation studies was reported in literature for bio-hydrogen production most of which are done by batch or fed-batch operation. Hydrogen formation rates and the yields in reported combined fermentations were compared with this study in Table 3.8. Hydrogen yields and rates differ due to utilization of different substrates, bacterial cultures, experimental conditions and operating modes. The highest H_2 yield by combined fermentation reported in literature is 7.1 molH_2 mol^{-1} glucose obtained by fed-batch operation Asada et al., (2009). There is only one literature report on continuous combined fermentation using periodic feeding and pure cultures Argun & Kargi, (2010c). The highest hydrogen yield obtained in this study (470 ml g^{-1} total sugar = 3.4 mol H_2 mol^{-1}glucose) is superior to the hydrogen yields reported in most of the combined fermentation studies (Argun et al., (2009a); Argun et al., (2009b); Argun & Kargi, (2010b); Argun & Kargi, (2010c); Fang et al., (2006); Liu et al., (2010); Kargi & Ozmihci, (2010); Ozmihci & Kargi, (2010a); Ozmihci & Kargi, (2010c); Xie et al., (2010);). High H_2 yield obtained in this study is probably due to by continuous operation using periodic feeding and effluent removal. Higher HRTs longer than 8 days may result in better hydrogen yields due to extended photo-fermentation. Continuous combined dark and photo-fermentation of acid hydrolyzed waste wheat starch by periodic feeding was proven to be an effective method yielding high hydrogen gas formation yields.

Table 3.8 Comparison of performances of combined fermentation processes for bio-hydrogen production.

Seed culture	Substrate	Light source	Light intensity	VHPR ($mLH_2L^{-1}h^{-1}$)	Yield (mol H_2 mol^{-1} glucose)	Operation Mode	Reference
Clostridium butyricum DSM 10702 & *Rhodobacter sphaeroides* DSM 158	Glucose (5 gL^{-1})	Tungsten	135 Wm^{-2}	–	0.86	Batch	Fang et al. (2006)
Clostridium butyricum & *Rhodopseudomonas faecalis* RLD-53	Glucose (9 gL^{-1})	Incandescent	4 klux	20.83	1.98	Batch	Liu et al. (2010)
Clostridium acidisoli & *Rhodobacter sphaeroides*	Sucrose (11.43gL^{-1})	–	4 klux	–	5.08	Batch	Sun et al. (2010)
Anaerobic sludge & *Rhodobater sphaeroide NRLL-1727*	Ground wheat starch (12.8 g L^{-1})	Tungsten and Halogen	6 ± 0.5 klux	1.40[a]	0.36	Batch	Ozmihci & Kargi (2010c)
Anaerobic sludge & *Rhodobacter* sp. RV	Ground wheat starch (4.3 g L^{-1})	Halogen	270 Wm^{-2}	6.70[a]	1.45	Batch	Argun & Kargi (2010b)
Anaerobic sludge & mixture of *Rhodobacter* sp.	Ground wheat starch (4.1 g L^{-1})	Fluorescent	9.5 klux	3.86[a]	1.16	Batch	Argun et al. (2009a)
Anaerobic sludge & mixture of *Rhodobacter* sp.	Ground wheat starch (3.9 g L^{-1})	Fluorescent	9.5 klux	7.12[a]	1.03	Batch	Argun et al. (2009b)
Lactobacillus delbrueckii NBRC 13953 & *Rhodobacter sphaeroides*-RV	Glucose (4.5 g L^{-1})	Halogen	0.19 µ Einstein $m^{-2}s^{-1}$	41.27[a]	7.1	Batch (Immobilized culture)	Asada et al. (2009)

Table 3.8 Continued

Clostridium butyricum & *Rhodo-pseudomonas faecalis* RLD-53	Glucose (6 g L^{-1})	Incandescent	8 klux	33.85	4.13	Batch (Immobilized)	Ding et al. (2009)
Ethanoligenens harbinense B49 & *Rhodopseudomonas faecalis* RLD-53	Glucose (6 g L^{-1})	Incandescent	4 klux	17.2	3.1	Batch (Immobilized)	Xie et al. (2010)
Clostridium butyricum & *Rhodobacter* sp. M-19	Starch (50 g L^{-1})	Incandescent	5 klux	16.50	6.6	Repeated Fed-Batch	Yokoi et al. (1998)
Anaerobic sludge and mixture of *Rhodobacter* sp.	Wheat powder solution (20 gdm^{-3})	Halogen	5 ± 0.5 klux	5.5 (mLH_2h^{-1})	0.43	Fed-Batch	Kargi & Ozmihci (2010)
Anaerobic sludge & *Rhodobacter* sp. (NRRL B-1727)	Ground wheat powder (10 g L^{-1})	Fluorescent	5 klux	18.1 (mLH_2h^{-1})	1.32	Fed-Batch	Ozmihci & Kargi (2010a)
Clostridium beijerinkii DSM-791 & *Rhodobacter* sp. RV	Ground wheat starch (5.0 g L^{-1})	Halogen and fluorescent	10 klux	5.95	0.6	Continuous by periodic feeding and effluent removal	Argun & Kargi (2010c)
Anaerobic sludge & *R. Shaeroides* (NRRL B-1727)	Sugar solution from acid hydrolyzed wheat starch (5.0 g L^{-1})	Halogen	5 klux	271.5 ml L^{-1} d^{-1} = 11.3 ml L^{-1} h^{-1}	370 ml H_2 g^{-1} total ugar = 3.4 mol H_2 mol^{-1} glucose	Continuous by periodic feeding and effluent removal	This study

a : Calculated by using the data in the relevant article.

CHAPTER FOUR
CONCLUSIONS

In batch dark fermentation experiments, hydrogen gas production from acid hydrolyzed ground wheat starch was investigated at different initial substrate and biomass concentrations. Heat treated anaerobic sludge was used as the inoculum culture. The initial substrate concentration substantially affected hydrogen gas yield and formation rate. Low substrate concentrations (TS < 10 g L^{-1}) resulted in the substrate limited conditions and high substrate concentrations (TS >15 g L^{-1}) lead to substrate and product inhibitions. Initial total sugar concentration of 10 g L^{-1} was determined to be the optimum, maximizing the rate and the yield of hydrogen gas formation. Dark fermentation of acid hydrolyzed ground wheat starch was proven to be more advantageous as compared to bacterial hydrolysis of boiled what starch during dark fermentation. Initial biomass concentration did not significantly affect the hydrogen gas evolution. Increasing biomass concentration resulted in substantial decreases in SHPR and the yield. The highest cumulative hydrogen gas formation (387 ml), hydrogen gas production rate (1221 ml H_2 g^{-1} cell h^{-1}) and the yield (1.52 mol H_2 mol^{-1} glucose) were obtained at the lowest biomass concentration 0.28 g L^{-1}. The most suitable X_o/S_o ratio, maximizing the yield and specific rate of formation was determined as $X_o/S_o = 0.037$ for bio-hydrogen production by batch dark fermentation of acid hydrolyzed wheat starch.

In continuous dark fermentation experiments with periodic feeding, the rate and yield of hydrogen gas production from acid hydrolyzed ground wheat varied with HRT due to variations in the composition and metabolic capabilities of the mixed bacterial culture. The highest hydrogen production rate (305 ml d^{-1}) was obtained at HRT = 6 h due to high total sugar loading rates at low HRTs. However, the yield was maximum (130 ml H_2 g^{-1} total sugar) at HRT = 24 h due to presence of effective hydrogen producing bacteria at this HRT.

Specific and volumetric rates of hydrogen production were also the highest at HRT = 6 h. Hydrogen formation was strongly related with the type and concentration of VFAs produced by the dominant bacterial culture at every HRT. The effluent VFA composition also varied with HRT in parallel to variations in composition of mixed bacterial culture. High acetate/butyrate ratios obtained at low HRTs yielded high hydrogen formation rates.

In continuous photo-fermentation experiments, dark fermentation effluent of acid hydrolyzed wheat starch was used for H_2 gas production by periodic feeding and effluent removal. Pure *Rhodobacter sphaeroides* (NRRL B-1727) culture was used and the fermenter was operated at different hydraulic residence times (HRT) between 1 and 10 days by adjusting the feeding rate. The highest hydrogen gas production rate (85 ml d^{-1}), specific and volumetric hydrogen formation rates were obtained when HRT was 4 days (96 h) while the highest hydrogen yield (1200 ml H_2 g^{-1} TVFA) was realized at HRT of 8 days. Increases in biomass concentration and the growth yield with HRT caused decreases in hydrogen formation at HRTs above 4 days. Optimum substrate (TVFA) loading rates yielding the highest hydrogen yield and formation rate were 0.32 g L^{-1} d^{-1} and 0.51 g L^{-1} d^{-1}, respectively. The highest hydrogen gas formation rate and the yield obtained in this study compared favorably with the literature studies.

Hydrogen gas production by continuous combined dark/photo fermentation of acid hydrolyzed wheat starch was realized by periodic feeding and effluent removal. A mixture of heat treated anaerobic sludge and *Rhodobacter sphaeroides* (NRRL-B 1727) were used as inocula. Effects of hydraulic residence time and total sugar loading rates on H_2 yield and formation rates were investigated at different hydraulic residence times (HRT) between 1 and 8 days. The highest hydrogen yield (470 ml g^{-1} total sugar = 3.4 mol H_2 mol^{-1}glucose) and daily hydrogen gas production (41 ml d^{-1}) were obtained at the HRT of 8 days. Hydrogen production at low HRTs (1-4 d) was mainly by dark fermentation, whereas at high HRTs (6-8 d) H_2 was produced by both the dark and light fermentations with increased H_2 yields. High biomass and low total volatile fatty acids (TVFA) concentrations at high HRTs were also indicators of

more effective H_2 gas production. The lowest total sugar loading rate of 0.625 $gL^{-1}d^{-1}$ resulted in the highest hydrogen yield and formation rate. Hydrogen gas yield obtained in continuous combined fermentation is superior to those obtained by batch combined fermentations due to continuous operation by periodic feeding and effluent removal.

Recommendations for future studies

Some of the recommendations for future studies are listed below:

- Anaerobic bacterial strains producing only acetic and butyric acids without lactic and propionic acids should be developed and used in dark fermentation.
- New strains or genetically modified photo-fermentative bacteria to tolerate high VFA and NH_4-N concentrations can be utilized.
- In order to eliminate inhibitory concentrations of substrate (VFAs) and product (H_2 gas) in fermentation, continuous removal of VFAs and hydrogen gas is recommended.
- More effective illumination and light distribution should be developed for photo and combined fermentations.
- To improve hydrogen formation optimization of environmental conditions and medium compositions is recommended.
- New technologies of reactors equipped with online pH, ORP, temperature, light control units, hydrogen impermeable pipelines (transfer of hydrogen gas, inlet and outlet) should be developed.

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A.1 Raw Data for Batch Dark Fermentation Experiments

A.1.1 Initial Substrate and Biomass Concentrations

Table A.1 Raw data for 3.9 gL^{-1} initial AHWS concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH2, i(%)	CH2, i-1(%)	Vw (mL)	VH2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	TVFA (mgL^{-1})
14.07.2009	19:00	0	240	370	0	0	0	0	7	-225	3920	134
15.07.2009	10:43	15,72	240	370	0,2080	0,2080	235	125,834	5,4	-332,8		
16.07.2009	10:38	39,63	233	377	0,2122	0,2122	20	133,1076	6,49	-401,3	330	2035
16.07.2009	18:18	47,30	227	383	0,2122	0,1836	0	134,3805	6,48	-397,5		
17.07.2009	10:21	63,35	221	389	0,2122	0,1528	0	135,6535	6,66	-403,8		
18.07.2009	14:36	91,60	213	397	0,2122	0,1322	0	137,3509	6,57	-412,1		

Table A.2 Raw data for 7.6 gL^{-1} initial AHWS concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH2, i(%)	CH_2, i-1(%)	Vw (mL)	VH2 (mL)	pH	ORP (mV)	Glucose (mgL^{-1})	TVFA (mgL^{-1})
30.06.2009	17:00	0	240	370	0	0	0	0,00	7	-310	7600	406
01.07.2009	11:20	23,76	230	380	0,3380	0,3380	400	263,60	5	-270,9		
02.07.2009	11:32	47,77	224	386	0,3793	0,3793	80	311,95	6,3	-368,2		

03.07.2009	11:41	71,78	218	392	0,3793	0,3376	10	317,60	6,3	-347,7	430	3125
04.07.2009	16:52	95,99	212	398	0,3793	0,2674	0	317,60	6,56	-342		
05.07.2009	13:16	119,84	206	404	0,3793	0,1489	0	317,60	6,65	-420,8		

Table A.3 Raw data for 10.0 gL^{-1} initial AHWS concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Glucose (mgL^{-1})**	**TVFA (mgL^{-1})**
14.07.2009	19:00	0	240	370	0	0	0	0,0	7	-225	9929	147
15.07.2009	10:27	17,45	240	370	0,3218	0,3218	430	257,4	4,72	-307		
16.07.2009	10:47	41,78	232	378	0,3356	0,3356	40	278,7	6,61	-392,5	206	3075
16.07.2009	18:13	49,22	226	384	0,3356	0,3186	0	280,7	6,72	-385,6		
17.07.2009	10:19	65,32	220	390	0,3356	0,2476	0	282,7	6,79	-422,8		
18.07.2009	14:38	93,63	212	398	0,3356	0,2086	0	285,4	6,5	-411,2		

Table A.4 Raw data for 15.0 gL^{-1} initial AHWS concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Glucose (mgL^{-1})**	**TVFA (mgL^{-1})**
14.07.2009	19:00	0	240	370	0	0	0	0,0	7	-225	15020	153
15.07.2009	10:05	17,08	240	370	0,3563	0,3563	480	302,9	5,4	-332,8		
16.07.2009	10:50	41,83	233	377	0,4412	0,4412	120	390,3	6,49	-401,3		
16.07.2009	18:03	49,05	227	383	0,4412	0,4046	25	403,0	6,48	-397,5	216	4410

17.07.2009	10:16	65,27	221	389	0,4412	0,3193	0	405,7	6,66	-403,8		
18.07.2009	14:40	93,67	213	397	0,4412	0,2075	0	409,2	6,7	-432,2		

Table A.5 Raw data for 22.0 gL^{-1} initial AHWS concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Glucose (mgL^{-1})**	**TVFA (mgL^{-1})**
14.07.2009	19:00	0	240	370	0	0	0	0,0	7	-225	27551	279
15.07.2009	08:46	15,77	240	370	0,4023	0,4023	120	197,1	5,4	-332,8		
16.07.2009	10:52	41,87	233	377	0,4927	0,4927	860	657,8	6,49	-401,3	1046	5935
16.07.2009	17:52	48,87	227	383	0,4927	0,4178	45	679,5	6,48	-397,5		
17.07.2009	10:09	65,15	221	389	0,4927	0,3435	30	692,8	6,66	-403,8		
18.07.2009	14:46	93,77	213	397	0,4927	0,2152	0	696,7	6,07	-420,2		

Table A.6 Raw data for 27.5 gL^{-1} initial AHWS concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Glucose (mgL^{-1})**	**TVFA (mgL^{-1})**
14.07.2009	19:00	0	240	370	0	0	0	0	7	-225	21932	298
15.07.2009	08:37	15,62	240	370	0,3787	0,3787	245	232,888	5,4	-332,8		
16.07.2009	10:43	41,72	233	377	0,5132	0,5132	200	388,893	6,49	-401,3		
16.07.2009	17:49	48,82	227	383	0,5132	0,4920	60	421,491	6,48	-397,5	1090	6205

17.07.2009	10:08	65,13	221	389	0,5132	0,4005	0	424,57	6,66	-403,8		
18.07.2009	14:48	93,80	213	397	0,5132	0,3706	0	428,675	6,28	-418,4		

Table A.7 Raw data for 0.28 gL^{-1} initial biomass concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Total sugar (mgL^{-1})**	**TVFA (mgL^{-1})**
29.07,.2009	20:30	0,00	275	335	0	0	0	0,0	7	-241		
30.07.2009	10:40	23,59	275	335	0,2741	0,2741	305	23,9	4,51	-258,6		
30.07.2009	18:10	23,90	272	338	0,4236	0,4236	160	48,2	5,83	-369,6		
31.07.2009	10:30	47,58	265	345	0,4286	0,4286	0	96,2	6,78	-392,7		
01.08.2009	13:30	71,71	264	346	0,4286	0,2906	0	168,2	6,75	-415,6		

Table A.8 Raw data for 0.55 gL^{-1} initial biomass concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Total sugar (mgL^{-1})**	**TVFA (mgL^{-1})**
29.07,.2009	20:42	0,00	275	335	0	0	0	0,00	7	-271		
30.07.2009	10:32	14,11	275	335	0,2844	0,2844	300	180,61	4,56	-326,1		
30.07.2009	18:15	21,83	273	337	0,4106	0,4106	205	307,86	5,98	-400,7		

31.07.2009	10:27	38,03	265	345	0,4106	0,3688	0	307,86	6,82	-387,7		
01.08.2009	13:27	65,03	264	346	0,4106	0,2829	0	307,86	6,99	-440,6		

Table A.9 Raw data for 0.72 gL^{-1} initial biomass concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Total sugar (mgL^{-1})**	**TVFA (mgL^{-1})**
29.07,.2009	20:42	0,00	275	335	0	0	0	0	7	-263		
30.07.2009	10:32	14,11	275	335	0,2577	0,2577	260	153,355	5	-409		
30.07.2009	18:15	21,83	273	337	0,4148	0,4148	220	298,041	5,76	-322,4		
31.07.2009	10:27	38,03	265	345	0,4148	0,3954	0	298,041	6,65	-398,3		
01.08.2009	13:25	65,00	264	346	0,4148	0,3218	0	298,041	6,73	-407,9		

Table A.10 Raw data for 0.83 gL^{-1} initial biomass concentration

Date	**Clock**	**Time (h)**	**Vl (mL)**	**Vg (mL)**	**CH2, i(%)**	**CH_2, i-1(%)**	**Vw (mL)**	**VH2 (mL)**	**pH**	**ORP (mV)**	**Total sugar (mgL^{-1})**	**TVFA (mgL^{-1})**
29.07,.2009	20:42	0,00	275	335	0	0	0	0	7	-254		
30.07.2009	10:32	14,11	275	335	0,2522	0,2522	255	148,807	4,88	-368,4		
30.07.2009	18:15	21,83	273	337	0,4133	0,4133	280	319,327	5,56	-332,8		
31.07.2009	10:27	38,03	265	345	0,4133	0,3891	0	319,327	6,67	-383,1		
01.08.2009	13:23	64,96	264	346	0,4133	0,3464	0	319,327	6,72	-398,6		

Table A.11 Raw data for 1.10 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH2, i(%)	CH_2, i-1(%)	Vw (mL)	VH2 (mL)	pH	ORP (mV)	Total sugar (mgL^{-1})	TVFA (mgL^{-1})
29.07,.2009	20:42	0,00	275	335	0	0	0	0,00	7	-244		
30.07.2009	10:32	23,58	275	335	0,2565	0,2565	240	147,51	5,05	-388,4		
30.07.2009	18:15	23,90	273	337	0,4020	0,4020	280	309,59	5,55	-303,4		
31.07.2009	10:27	47,57	265	345	0,4020	0,3322	0	309,59	6,79	-381,4		
01.08.2009	13:21	71,69	264	346	0,4020	0,2538	0	309,59	6,77	-436,5		

Table A.12 Raw data for 1.38 gL^{-1} initial biomass concentration

Date	Clock	Time (h)	Vl (mL)	Vg (mL)	CH2, i(%)	CH_2, i-1(%)	Vw (mL)	VH2 (mL)	pH	ORP (mV)	Total sugar (mgL^{-1})	TVFA (mgL^{-1})
29.07,.2009	20:42	0,00	275	335	0	0	0	0,00	7	-268		
30.07.2009	10:32	14,11	275	335	0,2842	0,2842	260	169,10	6,57	-158,4		
30.07.2009	18:15	21,83	273	337	0,4273	0,4273	235	318,31	7,2	-451,1		
31.07.2009	10:27	38,03	265	345	0,4273	0,3699	0	318,31	5,59	-235,9		
01.08.2009	13:19	64,90	264	346	0,4273	0,3300	0	318,31	4,65	-210		

A.2 Raw Data for Continuous Fermentation Experiments

A.2.1.Continuous Dark Fermentation

Table A.13 Raw data for HRT = 6 hours

DATE	HOUR	TIME (h)	Vs (ml)	Vg (ml)	H_2-1 (%)	Vw (ml)	H_2-1 (%)	VH_2 (ml/day)	pH	ORP	Glucose (mgL^{-1})	$Starch_o$ (mg/l)
11.02.2010	15:30	0	250	360	0	0	0	0,00	7			
12.02.2010	11:14	19,73	250	360	0,182	303	0,182	120,67	5,84	-311,2		
12.02.2010	16:46	25,27	250	360	0,1550	60	0,1550	65,10	5,81	-303,8		
13.02.2010	15:48	48,30	250	360	0,2740	280	0,2740	175,36	4,8	-215,8		
14.02.2010	13:55	70,42	250	360	0,1160	95	0,1160	52,78	5,18	-59,6		
15.02.2010	15:05	95,58	250	360	0,2590	320	0,2590	176,12	4,6	-170,3		
16.02.2010	15:59	120,48	250	360	0,3660	380	0,3660	270,84	5,3	-172,5		
17.02.2010	15:17	143,78	250	360	0,3460	350	0,3460	245,66	4,35	-233		
18.02.2010	16:54	169,40	250	360	0,2803	280	0,2803	179,39	4,43	-79	9951	10788
19.02.2010	15:12	191,70	250	360	0,3300	380	0,3300	244,20	4,98	-227,8	8952	9593
20.02.2010	15:37	216,12	250	360	0,3667	480	0,3667	308,03	5,28	-331,8	8165	8572
21.02.2010	16:19	240,82	250	360	0,3388	540	0,3388	304,92	5,94	-437,80	7952	8521
22.02.2010	16:17	264,78	250	360	0,3397	530	0,3397	302,33	6,06	-404,00	8225	8686
23.02.2010	16:12	288,70	250	360	0,3247	440	0,3247	259,76	6,26	-414,50		
24.02.2010	16:35	313,08	250	360	0,4145	200	0,4148	232,16	6,46	-133,60		

Table A.14 Raw data for HRT = 12 hours

DATE	HOUR	TIME (h)	Vs (ml)	Vg (ml)	H_2.1 (%)	Vw (ml)	H_2.1 (%)	VH_2 (ml/day)	pH	ORP	TS (mgL^{-1})	$Starch_o$ (mg/l)
				610	0	0	0	0				
11.02.2010	15:30	0	250	360	0	0	0	0	7			
12.02.2010	11:18	19,80	250	360	0,256	404	0,256	195,58	5,75	-333		
12.02.2010	16:54	25,40	250	360	0,2350	45	0,2350	95,18	5,74	-332		
13.02.2010	16:47	49,28	250	360	0,3200	540	0,3200	288,00	5,3	-252,8		
14.02.2010	14:01	70,52	250	360	0,1930	250	0,1930	117,73	4,7	-101,3		
15.02.2010	15:16	95,77	250	360	0,3210	400	0,3210	243,96	4,87	-202,3		
16.02.2010	16:07	120,62	250	360	0,2130	260	0,2130	132,06	4,97	-284,7		
17.02.2010	15:23	143,88	250	360	0,2660	325	0,2660	182,21	4,95	-316,8		
18.02.2010	17:05	169,58	250	360	0,1346	160	0,1346	69,99	4,49	-177,9	9951	10788
19.02.2010	15:19	191,82	250	360	0,2020	180	0,2020	109,08	4,86	-337,6	8952	9593
20.02.2010	15:45	216,25	250	360	0,3286	420	0,3286	256,31	5,04	-377,3	8165	8572
21.02.2010	17:12	241,70	250	360	0,2910	500	0,2910	250,26	5,39	-394,1	7952	8521
22.02.2010	16:24	264,90	250	360	0,3062	520	0,3062	269,46	5,9	-407,3	8225	8686
23.02.2010	16:15	288,75	250	360	0,2496	470	0,2496	207,17	6,32	-395,4		
24.02.2010	16:39	313,15	250	360	0,1153	240	0,1153	69,18	6,27	-136,8		

Table A.15 Raw data for HRT = 24 hours

DATE	HOUR	TIME (h)	Vs (ml)	Vg (ml)	H_2-1 (%)	Vw (ml)	H_2-1 (%)	VH_2 (ml/day)	pH	ORP	Total $Sugar_o$ (mgL^{-1})	$Starch_o$ (mg/l)
				610	0	0	0	0				
11.02.2010	15:30	0	250	360	0	0	0	0	7			
12.02.2010	11:21	19,85	250	360	0,24	384	0,256	184,70	5,68	-355,2		
12.02.2010	16:59	25,48	250	360	0,2220	65	0,2350	95,20	5,53	-343,7		
13.02.2010	16:35	49,08	250	360	0,1920	330	0,3200	174,72	5,41	-287,9		
14.02.2010	14:08	70,63	250	360	0,2000	280	0,1930	126,04	5,25	-217,6		
15.02.2010	15:23	95,88	250	360	0,1780	193	0,3210	126,03	4,52	-118,6		
16.02.2010	16:13	120,72	250	360	0,2630	290	0,2630	170,95	4,64	-208,2		
17.02.2010	15:32	144,03	250	360	0,2890	390	0,2890	216,75	4,62	-334,7		
18.02.2010	17:13	169,72	250	360	0,2623	360	0,2623	188,86	4,81	-314,1	9951	10788
19.02.2010	15:26	191,93	250	360	0,2810	440	0,2810	224,80	4,81	-368,1	8952	9593
20.02.2010	15:51	216,35	250	360	0,2926	460	0,2926	239,93	5,15	-400	8165	8572
21.02.2010	17:19	241,82	250	360	0,2971	480	0,2971	249,56	5,44	-263,1	7952	8521
22.02.2010	16:46	265,27	250	360	0,2735	400	0,2735	207,86	5,69	-389,8	8225	8686
23.02.2010	16:18	288,80	250	360	0,2552	380	0,2552	188,85	6,06	-338,5		
24.02.2010	16:42	313,20	250	360	0,1939	260	0,1939	120,218	6,09	-132		

Table A.16 Raw data for HRT =36 hours

DATE	*HOUR*	*TIME (h)*	*Vs (ml)*	*Vg (ml)*	*H_2.1 (%)*	*Vw (ml)*	*H_2.1 (%)*	*VH_2 (ml/day)*	*pH*	*ORP*	*TS_o (mgL^{-1})*	*$Starch_o$ (mg/l)*
				610	0	0	0	0				
11.02.2010	15:30	0	250	360	0	0	0	0	7			
12.02.2010	11:23	19,88	250	360	0,22	331	0,22	152,02	5,65	-344,8		
12.02.2010	17:31	26,02	250	360	0,2050	45	0,2050	83,03	5,64	-351,7		
13.02.2010	16:28	48,97	250	360	0,0051	180	0,0051	2,75	5,34	-299,6		
14.02.2010	14:14	70,73	250	360	0,0093	120	0,0093	4,46	5,9	-310		
15.02.2010	15:29	95,98	250	360	0,0371	180	0,0371	20,03	4,99	-200,8		
16.02.2010	16:29	120,98	250	360	0,1670	240	0,1670	100,20	4,93	-246		
17.02.2010	15:38	144,13	250	360	0,2300	380	0,2300	170,20	4,84	-162,7		
18.02.2010	17:29	169,98	250	360	0,0955	290	0,0955	62,08	5,09	-90,3	9951	10788
19.02.2010	16:06	192,60	250	360	0,1770	300	0,1770	116,82	5,06	-237,1	8952	9593
20.02.2010	16:17	216,78	250	360	0,1641	260	0,1641	101,74	5,08	-233,8	8165	8572
21.02.2010	18:18	242,80	250	360	0,1399	250	0,1399	85,34	5,11	-258,8	7952	8521
22.02.2010	17:04	265,57	250	360	0,1514	240	0,1514	90,86	5,26	-360,2	8225	8686
23.02.2010	16:20	288,83	250	360	0,1159	240	0,1159	69,54	5,66	-213,9		
24.02.2010	16:45	313,25	250	360	0,0892	230	0,0892	52,64	5,75	-123		

Table A.17 Raw data for HRT = 48 hours

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vw (ml)***	***H_2.1 (%)***	***VH_2 (ml/day)***	***pH***	***ORP***	***T S_o (mgL^{-1})***	***Starch$_o$ (mg/l)***
11.02.2010	15:30	0	250	360	0	0	0	0	7			
12.02.2010	11:28	19,97	250	360	0,24	417	0,24	186,48	5,76	-329,5		
12.02.2010	17:42	26,20	250	360	0,1840	45	0,1840	74,52	5,63	-317,3		
13.02.2010	16:13	48,72	250	360	0,0301	200	0,0301	16,86	5,36	-292,7		
14.02.2010	14:20	70,83	250	360	0,0865	140	0,0865	43,25	6,32	-322		
15.02.2010	16:04	96,57	250	360	0,0405	155	0,0405	20,86	5,45	-214,3		
16.02.2010	16:38	121,13	250	360	0,0484	160	0,0484	25,17	5,35	-228,6		
17.02.2010	16:04	144,57	250	360	0,0795	180	0,0795	42,93	4,88	-166,6		
18.02.2010	17:45	170,25	250	360	0,0926	180	0,0926	50,00	5,09	-55,8	9951	10788
19.02.2010	16:25	192,92	250	360	0,0612	250	0,0612	37,33	5,62	-190,1	8952	9593
20.02.2010	16:35	217,08	250	360	0,0942	240	0,0942	56,51	5,35	-197,8	8165	8572
21.02.2010	18:22	242,87	250	360	0,1028	200	0,1028	57,57	5,43	-286,7	7952	8521
22.02.2010	17:25	265,92	250	360	0,0871	200	0,0871	48,79	5,42	-320,7	8225	8686
23.02.2010	16:22	288,87	250	360	0,0950	200	0,0950	53,18	5,64	-198,9		
24.02.2010	16:47	313,28	250	360	0,0792	180	0,0792	42,76	5,78	-113,5		

Table A.18 Raw data for HRT = 60 hours

DATE	*HOUR*	*TIME (h)*	*Vs (ml)*	*Vg (ml)*	*H_2.1 (%)*	*Vw (ml)*	*H_2.1 (%)*	*VH_2 (ml/day)*	*pH*	*ORP*	*T S_o (mgL^{-1})*	*$Starch_o$ (mg/l)*
11.02.2010	15:30	0	250	360	0	0	0	0	7			
12.02.2010	11:39	19,97	250	360	0,17	307	0,17	113,39	6,06	-333,5		
12.02.2010	17:49	26,20	250	360	0,1360	45	0,1360	55,08	5,98	-321,7		
13.02.2010	16:08	48,72	250	360	0,0301	150	0,0301	15,35	5,38	-287,6		
14.02.2010	14:28	70,83	250	360	0,0865	85	0,0865	38,49	6,38	-311,9		
15.02.2010	16:28	96,57	250	360	0,0405	120	0,0405	19,44	5,51	-267,7		
16.02.2010	16:49	121,32	250	360	0,0098	125	0,0098	4,75	5,45	-282,9		
17.02.2010	16:11	144,68	250	360	0,0560	160	0,0560	29,12	5,05	-205,4		
18.02.2010	17:59	170,48	250	360	0,0848	215	0,0848	48,78	5,31	-187,3	9951	10788
19.02.2010	16:43	193,22	250	360	0,0831	120	0,0831	39,89	5,34	-236,5	8952	9593
20.02.2010	16:54	217,40	250	360	0,0776	140	0,0776	38,78	5,15	-277,1	8165	8572
21.02.2010	18:26	242,93	250	360	0,0498	160	0,0498	25,88	5,59	-239,8	7952	8521
22.02.2010	17:34	266,07	250	360	0,0809	140	0,0809	40,43	5,41	-284,3	8225	8686
23.02.2010	16:25	288,92	250	360	0,0662	140	0,0662	33,09	5,57	-217,1		
24.02.2010	16:50	313,33	250	360	0,0756	140	0,0756	37,82	5,71	-122,1		

A.2.2 Continuous Photo Fermentation

Table A.19 Raw data for HRT = 1 day

DATE	*HOUR*	*TIME (h)*	*Vs (ml)*	*Vg (ml)*	*H_2-1 (%)*	*Vw (ml)*	*H_2-1 (%)*	*VH_2 (ml/day)*	*pH*	*ORP*
11.05.2010	15:38	0	250	360	0,0218	0	0,0218	7,85	7,0	-74
12.05.2010	11:49	20,82	250	360	0,0343	0	0,0343	4,50	7,0	-165
13.05.2010	13:38	22,63	250	360	0,0541	3	0,0541	7,29	7,1	-97
15.05.2010	14:32	48,90	250	360	0,1060	7	0,1060	19,43	6,8	-48
17.05.2010	12:32	94,90	250	360	0,1220	5	0,1220	6,37	7,1	-83
20.05.2010	10:48	0,00	250	360	0,0948	0	0,0948	0,00	7,1	-120
21.05.2010	16:10	29,37	250	360	0,0851	0	0,0851	0,00	7,0	-34
22.05.2010	15:09	52,35	250	360	0,0866	0	0,0866	0,54	7,0	-55
23.05.2010	15:29	76,68	250	360	0,0000	0	0,0000	0,00	6,9	-37
24.05.2010	15:21	100,55	250	360	0,0000	0	0,0000	0,00	6,9	-49
25.05.2010	15:31	124,72	250	360	0,0000	0	0,0000	0,00	7,3	-216
26.05.2010	15:05	148,28	250	360	0,0000	0	0,0000	0,00	6,8	-131
27.05.2010	14:26	171,63	250	360	0,0032	0	0,0032	1,15	6,8	-142
28.05.2010	15:12	196,40	250	360	0,0000	0	0,0000	0,00	7,0	-83
29.05.2010	16:16	221,47	250	360	0,0000	0	0,0000	0,00	7,2	-15
30.05.2010	14:10	243,37	250	360	0,0029	0	0,0029	1,04	7,2	42
31.05.2010	15:41	268,88	250	360	0,0028	0	0,0028	0,00	7,1	35
01.06.2010	15:44	292,93	250	360	0,0000	0	0,0000	0,00	8,4	-86

02.06.2010	14:30	315,70	250	360	0,0000	0	0,0000	0,00	6,8	19
03.06.2010	16:24	341,60	250	360	0,0047	0	0,0047	1,69	7,0	-9
04.06.2010	14:11	363,38	250	360	0,0000	0	0,0000	0,00	7,4	-103
05.06.2010	15:56	389,13	250	360	0,0048	0	0,0048	1,73	9,0	-136
06.06.2010	16:22	413,57	250	360	0,0094	0	0,0094	1,66	9,3	-209
07.06.2010	14:51	435,97	250	360	0,0032	0	0,0032	1,00	7,3	-80
08.06.2010	16:39	461,78	250	360	0,0033	0	0,0033	0,04	6,9	-7
09.06.2010	15:55	485,07	250	360	0,0257	0	0,0257	2,00	7,8	-19
10.06.2010	16:47	510,13	250	360	0,1660	0	0,1660	2,00		

Table A.20 Raw data for HRT = 2 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2-1 (%)***	***Vw (ml)***	***H_2-1 (%)***	***VH_2 (ml/day)***	***pH***	***ORP***	***TVFAo (mgL^{-1})***	***NH4-No (mgL^{-1})***
11.05.2010	15:40	0	250	360	0,0251	2,5	0,0251	9,10	6,9	-111		
12.05.2010	11:58	20,19	250	360	0,0427	0	0,0427	6,34	7,0	-127		
13.05.2010	13:29	22,00	250	360	0,0760	6,5	0,0760	12,48	7,1	-98		
15.05.2010	14:14	48,90	250	360	0,1160	4	0,1160	14,86	6,9	-68		
17.05.2010	12:42	94,90	250	360	0,1120	0	0,1120	0,00	7,0	-81		
20.05.2010	10:50	0,00	250	360	0,1000	0	0,1000	0,00	7,1	-160		
21.05.2010	16:11	29,37	250	360	0,0943	0	0,0943	0,00	7,0	-115		
22.05.2010	15:12	52,35	250	360	0,0918	0	0,0918	0,00	7,0	-51		
23.05.2010	15:27	76,68	250	360	0,0000	0	0,0000	0,00	7,0	-47	2014	
24.05.2010	15:25	100,55	250	360	0,0026	0	0,0026	0,94	7,1	-9		

25.05.2010	15:34	124,72	250	360	0,0034	0	0,0034	0,29	7,2	-301		
26.05.2010	15:13	148,28	250	360	0,0000	0	0,0000	0,00	7,1	-141		
27.05.2010	14:32	171,63	250	360	0,0035	0	0,0035	1,26	7,1	-160		
28.05.2010	15:10	196,40	250	360	0,0000	0	0,0000	0,00	6,8	5	2032	7,89
29.05.2010	16:19	221,47	250	360	0,0000	0	0,0000	0,00	7,0	13		
30.05.2010	14:12	243,37	250	360	0,0000	0	0,0000	0,00	7,1	9	2344	
31.05.2010	15:37	268,88	250	360	0,0038	0	0,0038	1,37	7,4	48		
01.06.2010	15:48	292,93	250	360	0,0533	0	0,0533	17,82	7,3	-65		
02.06.2010	14:35	315,70	250	360	0,1650	0	0,1650	40,21	8,5	-38		
03.06.2010	16:21	341,60	250	360	0,3650	26	0,3650	81,49	7,9	-163		
04.06.2010	14:13	363,38	250	360	0,4500	8	0,4500	34,20	7,9	-189		
05.06.2010	16:01	389,13	250	360	0,5300	8	0,5300	33,04	7,5	-167		
06.06.2010	16:31	413,57	250	360	0,5470	40	0,5470	28,00	7,5	-217		
07.06.2010	14:51	436,05	250	360	0,5580	43	0,5580	27,95	7,3	-122		
08.06.2010	16:39	461,85	250	360	0,6250	5	0,6250	27,25	7,7	-128		
09.06.2010	15:55	485,12	250	360	0,7510	9	0,7510	52,12	7,7	-23		
10.06.2010	16:47	509,98	250	360	0,6540	0	0,6540	0,00				

Table A.21 Raw data for HRT = 4 days

DATE	*HOUR*	*TIME (h)*	*Vs (ml)*	*Vg (ml)*	*H_2.1 (%)*	*Vtotal gas (ml)*	*H_2.1 (%)*	*H_2 Production (ml/day)*	*pH*	*ORP*	*TVFAo (gL^{-1})*	*NH4-No (mgL^{-1})*
11.05.2010	15:41	0	250	360	0,0207	0	0,0207	7	7,0	-93		
12.05.2010	11:54	20,22	250	360	0,0281	0	0,0281	3	7,0	-127		
13.05.2010	13:35	21,90	250	360	0,0621	4	0,0621	12	7,0	-102		
15.05.2010	14:22	48,79	250	360	0,1010	6	0,1010	15	6,9	-74		
17.05.2010	12:39	95,07	250	360	0,0962	0	0,0962	0	6,9	-77		
20.05.2010	10:52	0,00	250	360	0,0862	0	0,0862	0	7,0	-112		
21.05.2010	16:16	29,40	250	360	0,0802	0	0,0802	0	7,0	-115		
22.05.2010	16:15	53,38	250	360	0,0847	0	0,0847	2	7,0	-47		
23.05.2010	15:25	76,55	250	360	0,0000	0	0,0000	0	7,0	-56	2,014	
24.05.2010	15:28	100,60	250	360	0,0000	0	0,0000	0	7,2	-7		
25.05.2010	15:35	124,71	250	360	0,0000	0	0,0000	0	7,1	-312		
26.05.2010	15:11	148,31	250	360	0,0043	0	0,0043	2	7,2	-140		
27.05.2010	14:33	171,68	250	360	0,0030	0	0,0030	0	6,9	-163		
28.05.2010	15:07	196,25	250	360	0,0000	0	0,0000	0	7,0	-9	2,032	7,89
29.05.2010	16:21	221,48	250	360	0,0257	0	0,0257	9	6,9	-46		
30.05.2010	14:15	243,38	250	360	0,0896	0	0,0896	23	7,0	-51	2,344	
31.05.2010	15:35	268,71	250	360	0,1430	0,5	0,1430	19	7,0	-30		
01.06.2010	15:53	293,01	250	360	0,2620	45,5	0,2620	55	7,0	-72		
02.06.2010	14:41	315,81	250	360	0,3560	15	0,3560	39	7,2	-53		
03.06.2010	16:16	341,40	250	360	0,4260	13	0,4260	31	7,2	-81		
04.06.2010	14:23	363,51	250	360	0,4500	14	0,4500	15	7,2	-167		

05.06.2010	16:05	389,21	250	360	0,5400	10	0,5400	38	7,3	-147		
06.06.2010	16:37	413,75	250	360	0,5810	70	0,5810	55	7,2	-142		
07.06.2010	14:57	436,08	250	360	0,6250	42	0,6250	42	7,6	-191		
08.06.2010	16:44	461,86	250	360	0,7230	29	0,7230	56	7,6	-140		
09.06.2010	16:00	485,13	250	360	0,7470	65	0,7470	57	7,5	-20		
10.06.2010	16:39	509,78	250	360	0,7400	100	0,7400	74	7,5	-185		
11.06.2010	16:21	533,48	250	360	0,7910	50	0,7910	58	7,4	-166		
12.06.2010	16:17	557,41	250	360	0,7830	100	0,7830	78	7,4	-259		
13.06.2010	15:54	581,03	250	360	0,8010	80	0,8010	71	7,3	-197		
14.06.2010	17:17	606,41	250	360	0,8480	80	0,8480	85	7,4	-126	2,032	
15.06.2010	16:17	629,41	250	360	0,8500	80	0,8500	69	7,1	-338		
16.06.2010	18:12	655,33	250	360	0,3380	65	0,3380	86	7,3	-349	2,344	
17.06.2010	15:39	676,78	250	360	0,4500	95	0,4500	83	7,2	-334		
18.06.2010	18:53	704,01	250	360	0,4990	90	0,4990	63	7,2	-408		
19.06.2010	18:35	727,71	250	360	0,5800	80	0,5800	76	7,3	-435		
20.06.2010	18:47	751,91	250	360	0,57	65	0,57	37	7,2	-328		
21.06.2010	18:34	775,70	250	360	0,536	75	0,536	40	7,3	-185		

Table A.22 Raw data for HRT = 6 days

DATE	*HOUR*	*TIME (h)*	*Vs (ml)*	*Vg (ml)*	*H_2-1 (%)*	*Vw (ml)*	*H_2-1 (%)*	*VH_2 (ml/day)*	*pH*	*ORP*	*TVFAo (mgL^{-1})*	*NH4-No (mgL^{-1})*
11.05.2010	15:43	0	250	360	0,0234	3,5	0,0234	8,51	7,0	-103		
12.05.2010	11:56	20,21	250	360	0,3670	0	0,3670	123,70	7,1	-138		
13.05.2010	13:32	21,81	250	360	0,0720	4,5	0,0720	0,32	7,0	-103		
15.05.2010	14:25	48,89	250	360	0,1000	3	0,1000	10,38	6,8	-74		
17.05.2010	12:37	95,09	250	360	0,1040	2	0,1040	1,65	6,9	-72		
20.05.2010	10:55	0,00	250	360	0,0767	0	0,0767	0,00	7,0	-62		
21.05.2010	16:19	29,40	250	360	0,0775	2	0,0775	0,44	7,0	-62		
22.05.2010	15:17	52,36	250	360	0,0791	0	0,0791	0,58	7,0	-43		
23.05.2010	15:23	76,46	250	360	0,0000	0	0,0000	0,00	7,0	-32	2014	
24.05.2010	15:30	100,58	250	360	0,0026	0	0,0026	0,94	7,2	-91		
25.05.2010	15:37	124,70	250	360	0,0030	0	0,0030	0,14	7,1	-144		
26.05.2010	15:08	148,21	250	360	0,0046	0	0,0046	0,58	7,0	-141		
27.05.2010	14:35	171,66	250	360	0,0067	0	0,0067	0,76	6,9	-167		
28.05.2010	15:06	196,18	250	360	0,0030	0	0,0030	0,00	7,0	19	2032	7,89
29.05.2010	16:22	221,45	250	360	0,0026	0	0,0026	0,00	6,9	2		
30.05.2010	14:17	243,36	250	360	0,0026	0	0,0026	0,00	6,8	6	2344	
31.05.2010	15:32	268,61	250	360	0,0044	0	0,0044	0,65	6,7	25		
01.06.2010	15:56	293,01	250	360	0,0046	0	0,0046	0,07	6,8	19		
02.06.2010	14:50	315,91	250	360	0,0030	0	0,0030	0,00	6,8	-10		
03.06.2010	16:12	341,28	250	360	0,0056	0	0,0056	0,94	7,1	-3		

04.06.2010	14:25	363,50	250	360	0,0035	0	0,0035	0,00	7,0	-104		
05.06.2010	16:08	389,21	250	360	0,0397	0	0,0397	13,03	7,2	-136		
06.06.2010	16:42	413,78	250	360	0,1490	0	0,1490	39,35	7,3	-115		
07.06.2010	15:03	436,13	250	360	0,2420	5	0,2420	34,69	7,6	-162		
08.06.2010	16:48	461,88	250	360	0,3850	11	0,3850	55,72	7,8	-114		
09.06.2010	16:03	485,13	250	360	0,4620	50	0,4620	50,82	7,7	-124		
10.06.2010	16:37	509,70	250	360	0,5340	80	0,5340	68,64	7,7	-192		
11.06.2010	16:17	533,36	250	360	0,5600	85	0,5600	56,96	7,6	-235		
12.06.2010	16:15	557,33	250	360	0,5870	100	0,5870	68,42	7,6	-250		
13.06.2010	15:49	580,90	250	360	0,6230	80	0,6230	62,80	7,6	-221		
14.06.2010	17:15	589,81	250	360	0,6670	70	0,6670	62,53	7,6	-272		
15.06.2010	16:14	629,31	250	360	0,6870	20	0,6870	20,94	7,3	-203		
16.06.2010	18:04	655,15	250	360	0,1520	20	0,1520	19,96	7,5	-217		
17.06.2010	17:22	678,45	250	360	0,2510	20	0,2510	40,66	7,4	-183		
18.06.2010	18:50	703,91	250	360	0,2890	15	0,2890	18,02	7,5	-141		
19.06.2010	18:33	727,63	250	360	0,298	0	0,298	3,24	7,6	-266		
20.06.2010	18:45	751,83	250	360	0,377	20	0,377	35,98	7,3	-183		
21.06.2010	18:37	775,70	250	360	0,391	30	0,391	16,77	7,2	-249		
22.06.2010	16:23	797,46	250	360	0,425	15	0,425	18,62	7,6	-397		
23.06.2010	17:53	822,96	250	360	0,41	40	0,41	16,40	7,5	-222		
24.06.2010	16:58	846,05	250	360	0,402	30	0,402	12,06	7,6			
25.06.2010	15:41	868,76	250	360	0,558	25	0,558	70,11	7,4			
26.06.2010	16:02	893,11	250	360	0,537	40	0,537	21,48	7,5			
27.06.2010	19:24	920,48	250	360	0,527	20	0,527	10,54	7,5			
28.06.2010	16:31	941,60	250	360	0,449	40	0,449	17,96	7,4			

29.06.2010	16:58	966,05	250	360	0,471	20	0,471	17,34	7,3	-210		
30.06.2010	17:03	990,13	250	360	0,56	20	0,56	43,24	7,3	-215		
01.07.2010	16:54	1013,98	250	360	0,481	25	0,481	12,03	7,6	-216		
02.07.2010	15:52	1036,95	250	360	0,641	10	0,641	64,01	7,5	-207		
03.07.2010	17:38	1062,71	250	360	0,64	20	0,64	12,80	7,49	-216		
04.07.2010	17:07	1086,20	250	360	0,625	20	0,625	12,50	7,5	-225		
05.07.2010	17:26	1110,51	250	360	0,53	25	0,53	13,25	7,37	-214		
06.07.2010	16:28	1133,55	250	360	0,606	15	0,606	36,45	7,5	-204		
07.07.2010	17:02	1158,11	250	360	0,737	65	0,737	95,07	7,5			
08.07.2010	17:02	1182,11	250	360	0,654	20	0,654	13,08	7,5			
09.07.2010	15:03	1204,13	250	360	0,739	13	0,739	40,21	7,17			
10.07.2010	13:05	1226,16	250	360	0,758	35	0,758	33,37	7,12			
11.07.2010	15:06	1252,18	250	360	0,682	35	0,682	23,87	7,37			
12.07.2010	16:35	1277,66	250	360	0,646	20	0,646	12,92	7,37			
13.07.2010	16:36	1301,68	250	360	0,676	0	0,676	10,80	7,28			

Table A.23 Raw data for HRT = 8 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vw (ml)***	***H_2.1 (%)***	***VH_2 (ml/day)***	***pH***	***ORP***	***TVFAo (mgL^{-1})***	***NH4-No (mgL^{-1})***
11.05.2010	15:46	0	250	360	0,0187	2	0,0187	6,77	6,9	-103		
12.05.2010	11:52	20,10	250	360	0,0283	0	0,0283	3,46	7,0	-138		
13.05.2010	13:40	21,90	250	360	0,0434	0	0,0434	5,44	7,0	-103		

15.05.2010	14:34	48,90	250	360	0,0688	3	0,0688	9,35	6,8	-74		
17.05.2010	12:35	94,91	250	360	0,0912	5	0,0912	8,52	6,8	-72		
20.05.2010	10:57	0,00	250	360	0,1120	4	0,1120	7,94	6,9	-62		
21.05.2010	16:22	29,42	250	360	0,0984	0	0,0984	0,00	7,0	-62		
22.05.2010	15:19	52,37	250	360	0,0924	0	0,0924	0,00	6,9	-43		
23.05.2010	15:21	76,40	250	360	0,0028	0	0,0028	0,00	7,0	-32	2014	
24.05.2010	15:33	100,97	250	360	0,0025	0	0,0025	0,00	7,2	-91		
25.05.2010	15:38	124,68	250	360	0,0027	0	0,0027	0,07	7,2	-144		
26.05.2010	15:03	148,10	250	360	0,0027	0	0,0027	0,00	7,0	-141		
27.05.2010	14:37	171,67	250	360	0,0071	0	0,0071	1,58	7,2	-167		
28.05.2010	15:04	196,12	250	360	0,0593	0	0,0593	18,79	7,5	19	2032	7,89
29.05.2010	16:25	221,47	250	360	0,2260	20	0,2260	64,53	7,4	2		
30.05.2010	14:20	243,38	250	360	0,2670	13,5	0,2670	18,36	7,1	6	2344	
31.05.2010	15:29	268,53	250	360	0,1730	7	0,1730	1,21	7,0	25		
01.06.2010	16:00	293,05	250	360	0,3250	25	0,3250	62,85	7,1	19		
02.06.2010	15:01	316,07	250	360	0,4050	17	0,4050	35,69	7,1	-10		
03.06.2010	16:09	341,20	250	360	0,4620	10	0,4620	25,14	7,1	-3		
04.06.2010	14:31	363,57	250	360	0,4880	45	0,4880	31,32	7,1	-104		
05.06.2010	16:12	389,25	250	360	0,5450	70	0,5450	58,67	7,2	-136		
06.06.2010	16:47	413,83	250	360	0,5880	25	0,5880	30,18	7,1	-115		
07.06.2010	15:07	436,17	250	360	0,6180	7	0,6180	15,13	7,4	-202		
08.06.2010	16:55	461,97	250	360	0,7820	12	0,7820	68,42	7,5	-124		
09.06.2010	16:07	485,17	250	360	0,8570	50	0,8570	69,85	7,3	-168		
10.06.2010	16:33	509,60	250	360	0,7230	65	0,7230	47,00	7,5	-212		
11.06.2010	16:14	533,28	250	360	0,7490	80	0,7490	69,28	7,5	-203		

12.06.2010	16:12	557,42	250	360	0,7860	60	0,7860	60,48	7,4	-174		
13.06.2010	15:45	580,80	250	360	0,7750	70	0,7750	54,25	7,4	-256		
14.06.2010	17:12	606,25	250	360	0,8200	80	0,8200	81,80	7,4	-230		
15.06.2010	16:10	629,22	250	360	0,8110	50	0,8110	40,55	7,1	-180		
16.06.2010	18:00	655,05	250	360	0,3430	45	0,3430	59,36	7,3	-107		
17.06.2010	17:20	678,38	250	360	0,4310	45	0,4310	51,08	7,4	-238		
18.06.2010	18:46	703,82	250	360	0,49	45	0,49	43,29	7,3	-180		
19.06.2010	18:30	727,55	250	360	0,523	40	0,523	32,80	7,2	-202		
20.06.2010	18:41	751,73	250	360	0,478	60	0,478	28,68	7,1	-263		
21.06.2010	18:40	775,72	250	360	0,635	80	0,635	107,32	7,6	-232		
22.06.2010	16:26	797,48	250	360	0,679	45	0,679	46,40	7,1	-304		
23.06.2010	17:48	822,85	250	360	0,691	80	0,691	59,60	7,0	-243		
24.06.2010	16:55	845,97	250	360	0,686	65	0,686	44,59	7,0			
25.06.2010	15,:38	868,63	250	360	0,743	65	0,743	68,82	7,0			
26.06.2010	16:00	893,05	250	360	0,774	65	0,774	61,47	7,1			
27.06.2010	19,22	920,45	250	360	0,85	60	0,85	78,36	7,1			
28.06.2010	16:23	941,43	250	360	0,758	30	0,758	22,74	7,2			
29.06.2010	16:56	965,98	250	360	0,348	40	0,348	91,32	7,2	-225		
30.06.2010	17:00	990,05	250	360	0,401	50	0,401	39,13	7,2	-220		
01.07.2010	16:58	1014,02	250	360	0,42	50	0,42	27,84	7,3	-224		
02.07.2010	15:49	1036,87	250	360	0,578	55	0,578	88,67	7,25	-204		
03.07.2010	17:36	1062,65	250	360	0,613	50	0,613	43,25	7,2	-211		
04.07.2010	17:10	1086,22	250	360	0,61	45	0,61	27,45	7,2	-193		
05.07.2010	17:23	1110,43	250	360	0,564	50	0,564	28,20	7,15	-221		
06.07.2010	16:24	1133,45	250	360	0,51	55	0,51	28,05	7,02	-215		

07.07.2010	16:59	1158,03	250	360	0,708	20	0,708	85,44	7,1			
08.07.2010	17:04	1182,12	250	360	0,739	65	0,739	59,20	7,39			
09.07.2010	15:07	1204,17	250	360	0,788	93	0,788	90,92	6,98			
10.07.2010	13:03	1226,10	250	360	0,809	75	0,809	68,24	6,94			
11.07.2010	15:08	1252,18	250	360	0,819	95	0,819	81,41	6,97			
12.07.2010	16:37	1277,67	250	360	0,777	85	0,777	66,05	7,07			
13.07.2010	16:34	1301,62	250	360	0,802	80	0,802	73,16	7,03			
14.07.2010	15:48	1324,85	250	360	0,848	80	0,848	84,40	6,86			
15.07.2010	15:52	1348,92	250	360	0,821	85	0,821	69,79	6,81			
16.07.2010	12:50	1369,88	251	359	0,811	85	0,811	68,94	6,92			
17.07.2010	12:42	1393,75	252	358	0,853	65	0,853	70,48	6,71	-235		
18.07.2010	14:53	1419,93	253	357	0,85	80	0,85	68,00	6,66	-220		
19.07.2010	17:10	1446,22	254	356	0,831	90	0,831	74,79	6,81	-218		
20.07.2010	15:51	1468,90	255	355	0,833	65	0,833	54,86	6,81	-136		
21.07.2010	17:18	1494,35	256	354	0,859	75	0,859	73,63	6,88	-127		
22.07.2010	16:34	1517,62	257	353	0,863	85	0,863	74,77	6,89	-140		
23.07.2010	16:11	1541,23	258	352	0,895	65	0,895	69,44	6,8	-160		
24.07.2010	16:32	1565,58	259	351	0,903	70	0,903	66,02	6,89	-328		
25.07.2010	16:50	1589,88	260	350	0,895	80	0,895	71,60	6,9	-53		
26.07.2010	17:09	1614,20	261	349	0,859	80	0,859	68,72	7,01	-215		
27.07.2010	13:31	1634,57	262	348	0,863	80	0,863	70,43	6,95			
28.07.2010	15:29	1660,53	263	347	0,911	60	0,911	71,32				

Table A.24 Raw data for HRT = 10 days

DATE	*HOUR*	*TIME (h)*	*Vs (ml)*	*Vg (ml)*	*H_2.1 (%)*	*Vw (ml)*	*H_2.1 (%)*	*VH_2 (ml/day)*	*pH*	*ORP*	*TVFAo (mgL^{-1})*	*NH4-No (mgL^{-1})*
11.05.2010	15:47	0	250	360	0,029	0	0,029	10,55	6,9	-121		
12.05.2010	11:51	20	250	360	0,043	0	0,043	4,90	7,0	-139		
13.05.2010	13:42	22	250	360	0,064	3	0,064	7,61	7,0	-116		
15.05.2010	14:27	49	250	360	0,112	8	0,112	18,36	6,8	-71		
17.05.2010	12:29	95	250	360	0,119	10	0,119	3,71	6,8	-64		
20.05.2010	10:58	0	250	360	0,122	3	0,122	1,45	6,9	-134		
21.05.2010	16:25	29	250	360	0,100	0	0,100	0,00	6,8	-63		
22.05.2010	15:21	52	250	360	0,108	0	0,108	2,88	6,9	-30		
23.05.2010	15:19	76	250	360	0,004	2	0,004	0,01	7,1	-73	2014	
24.05.2010	15:35	101	250	360	0,003	0	0,003	0,00	7,1	-57		
25.05.2010	15:40	125	250	360	0,000	0	0,000	0,00	7,3	-17		
26.05.2010	14:59	148	250	360	0,003	0	0,003	0,97	7,2	-66		
27.05.2010	14:39	172	250	360	0,004	0	0,004	0,61	7,3	-90		
28.05.2010	15:01	196	250	360	0,070	0	0,070	23,65	7,8	-275	2032	7,89
29.05.2010	16:28	222	250	360	0,200	21,5	0,200	51,06	7,2	-187		
30.05.2010	16:23	245	250	360	0,211	8	0,211	5,65	7,3	-111	2344	
31.05.2010	15:26	268	250	360	0,200	0	0,200	0,00	7,3	-178		
01.06.2010	16:04	293	250	360	0,276	16,5	0,276	31,91	7,1	-125		
02.06.2010	14:59	316	250	360	0,341	20	0,341	30,22	7,2	-153		
03.06.2010	16:06	341	250	360	0,404	9	0,404	26,32	7,2	-128		

04.06.2010	14:34	364	250	360	0,455	40	0,455	36,56	7,3	-153		
05.06.2010	16:16	389	250	360	0,509	40	0,509	39,80	7,3	-151		
06.06.2010	16:51	414	250	360	0,521	40	0,521	25,16	7,2	-150		
07.06.2010	15:12	436	250	360	0,567	25	0,567	30,74	7,7	-235		
08.06.2010	16:59	462	250	360	0,832	16	0,832	108,71	7,5	-122		
09.06.2010	16:11	485	250	360	0,654	50	0,654	32,70	7,4	-168		
10.06.2010	16:24	509	250	360	0,709	60	0,709	62,34	7,7	-107		
11.06.2010	16:10	533	250	360	0,719	50	0,719	39,55	7,7	-80		
12.06.2010	16:09	557	250	360	0,727	50	0,727	39,23	7,7	-241		
13.06.2010	15:42	581	250	360	0,710	55	0,710	39,05	7,6	-257		
14.06.2010	17:10	606	250	360	0,769	45	0,769	55,85	7,7	-323		
15.06.2010	16:07	629	250	360	0,769	25	0,769	19,23	7,4	-263		
16.06.2010	17:58	655	250	360	0,185	50	0,185	38,05	7,6	-153		
17.06.2010	17:16	678	250	360	0,256	27	0,256	32,47	7,4	-151		
18.06.2010	18:43	704	250	360	0,255	0	0,255	0,00	7,6	-166		
19.06.2010	18:28	728	250	360	0,263	0	0,263	2,88	7,7	-143		
20.06.2010	18:39	752	250	360	0,303	0	0,303	14,40	7,4	-124		
21.06.2010	18:44	776	250	360	0,307	20	0,307	7,58	7,7	-363		
22.06.2010	16:29	798	250	360	0,340	20	0,340	18,68	7,6	-225		
23.06.2010	17:44	823	250	360	0,341	15	0,341	5,48	7,7	-42		
24.06.2010	16:51	846	250	360	0,419	0	0,419	28,08	7,5			
25.06.2010	15:34	869	250	360	0,348	0	0,348	0,00	7,5			
26.06.2010	15:53	893	250	360	0,347	0	0,347	0,00	7,5			
27.06.2010	19:20	920	250	360	0,443	3	0,443	35,89	7,6			
28.06.2010	16:20	941	250	360	0,391	1	0,391	0,39	7,4			

29.06.2010	16:51	966	250	360	0,413	2	0,413	8,75	7,0	-175		
30.06.2010	16:59	990	250	360	0,307	0,5	0,307	0,15	6,8	-185		
01.07.2010	17:03	1014	250	360	0,351	0	0,351	15,84	7,4	-194		
02.07.2010	15:47	1037	250	360	0,414	0	0,414	22,68	7,4	-170		
03.07.2010	17:32	1063	250	360	0,417	0	0,417	1,08	7,5	-182		
04.07.2010	17:12	1086	250	360	0,408	0	0,408	0,00	7,2	-175		
05.07.2010	17:20	1110	250	360	0,362	0	0,362	0,00	7,0	-165		
06.07.2010	16:20	1133	250	360	0,379	1	0,379	6,50	7,3	-178		
07.07.2010	16:57	1158	250	360	0,525	7	0,525	56,24	7,3			
08.07.2010	16:54	1182	250	360	0,528	20	0,528	11,64	7,1			
09.07.2010	15:09	1204	250	360	0,563	50	0,563	40,75	6,9			
10.07.2010	13:01	1226	250	360	0,653	40	0,653	58,52	7,0			
11.07.2010	15:10	1252	250	360	0,655	45	0,655	30,20	7,3			
12.07.2010	16:38	1278	250	360	0,532	45	0,532	23,94	7,3			
13.07.2010	16:24	1301	250	360	0,380	20	0,38	7,60	7,1			
14.07.2010	15:43	1325	250	360	0,607	40	0,607	106,00	7,1			
15.07.2010	15:44	1349	250	360	0,688	40	0,688	56,68	7,0			
16.07.2010	12:52	1370	250	360	0,777	40	0,777	63,12	6,8			
17.07.2010	12:45	1394	250	360	0,811	23	0,811	30,89	7,3	-284		
18.07.2010	14:51	1420	250	360	0,818	35	0,818	31,15	6,9	-199		
19.07.2010	17:08	1446	250	360	0,887	40	0,887	60,32	6,8	-53		
20.07.2010	15:47	1469	251	359	0,898	35	0,898	35,38	7,21	-170		
21.07.2010	17:16	1494	252	358	0,837	40	0,837	33,48	6,81	-125		
22.07.2010	16:32	1518	253	357	0,82	40	0,82	32,80	7,15	-144		
23.07.2010	16:06	1541	254	356	0,87	20	0,87	35,20	7,04	-184		

24.07.2010	16:28	1566	255	355	0,869	35	0,869	30,42	7,03	-347		
25.07.2010	16:47	1590	256	354	0,887	40	0,887	41,85	7,11	-257		
26.07.2010	17:07	1614	257	353	0,855	40	0,855	34,20	7,06	-89		
27.07.2010	13:29	1635	258	352	0,755	40	0,755	30,20	7,17	-105		
28.07.2010	15:27	1660	259	351	0,92	7	0,92	64,36	7,13	-58		

A.2.3 Continuous Combined Fermentation

Table A.25 Raw data for HRT = 1 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vw (ml)***	***H_2.1 (%)***	***VH_2 (ml/day)***	***pH***	***ORP***
				310	0	0	0	0		
05.08.2010	17:00	0	150	160	0,000	0	0,000	0,00	7,00	
06.08.2010	18:06	25	150	160	0,082	2	0,082	13,33	6,1	-41
07.08.2010	15:30	47	150	160	0,053	0	0,053	0,00	6,5	-136
08.08.2010	15:42	71	150	160	0,124	10	0,124	12,65	6,6	-97
09.08.2010	16:13	95	150	160	0,206	17	0,206	16,62	6,7	-219
10.08.2010	11:10	114	150	160	0,306	30	0,306	25,18	6,6	-201
11.08.2010	13:25	140	150	160	0,380	35	0,380	25,14	6,6	-361
12.08.2010	11:36	163	150	160	0,382	65	0,382	25,15	6,2	-337
13.08.2010	16:22	191	150	160	0,363	60	0,363	21,78	6,1	-340
14.08.2010	16:26	215	150	160	0,317	65	0,317	20,61	6,9	-367
15.08.2010	17:32	241	150	160	0,269	30	0,269	8,07	6,8	-342

16.08.2010	19:15	266	150	160	0,217	25	0,217	5,43	6,7	-314

Table A.26 Raw data for HRT = 2 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vw (ml)***	***H_2.1 (%)***	***VH_2 (ml/day)***	***pH***	***ORP***
				310	0	0	0	0		
05.08.2010	17:00	0	150	160	0,000	0	0,000	0,00	7,00	
06.08.2010	18:10	25	150	160	0,065	5	0,065	10,73	6,1	-41
07.08.2010	15:33	47	150	160	0,085	0	0,085	3,22	6,5	-136
08.08.2010	15:45	71	150	160	0,119	10	0,119	6,61	6,6	-97
09.08.2010	16:16	95	150	160	0,107	0	0,107	0,00	6,7	-219
10.08.2010	11:14	114	150	160	0,098	0	0,098	0,00	6,6	-201
11.08.2010	13:29	140	150	160	0,094	0	0,094	0,00	6,6	-361
12.08.2010	11:34	163	150	160	0,105	35	0,105	5,37	6,2	-337
13.08.2010	16:25	191	150	160	0,092	30	0,092	2,75	6,1	-340
14.08.2010	16:29	215	150	160	0,088	15	0,088	1,32	6,9	-367
15.08.2010	17:36	241	150	160	0,080	20	0,080	1,59	6,8	-342
16.08.2010	19:17	266	150	160	0,071	20	0,071	1,43	6,7	-314
17.08.2010	16:19	287	150	160	0,064	45	0,064	2,86		
18.08.2010	16:19	311	150	160	0,058	20	0,058	1,16		
19.08.2010	16:20	335	150	160	0,058	25	0,058	1,49		
20.08.2010	16:31	360	150	160	0,056	25	0,056	1,41		
21.08.2010	16:31	384	150	160	0,079	10	0,079	4,46		

22.08.2010	16:14	407	150	160	0,099	10	0,099	4,16		
23.08.2010	16:57	432	150	160	0,138	0	0,138	6,26		
24.08.2010	13:21	452	150	160	0,169	5	0,169	5,81		
25.08.2010	16:34	480	150	160	0,199	5	0,199	5,80		
26.08.2010	14:00	501	150	160	0,204	15	0,204	3,86		
27.08.2010	14:53	526	150	160	0,222	5	0,222	3,99		
28.08.2010	16:14	551	150	160	0,181	15	0,181	2,72		
29.08.2010	17:30	577	150	160	0,168	10	0,168	1,68		
30.08.2010	20:05	603	150	160	0,139	0	0,139	0,00		

Table A.27 Raw data for HRT = 4 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vw (ml)***	***H_2.1 (%)***	***VH_2 (ml/day)***	***pH***	***ORP***
				310	0	0	0	0		
05.08.2010	17:00	0	150	160	0,000	0	0,000	0,00	7,00	
06.08.2010	18:10	25	150	160	0,047	0	0,047	7,46	6,1	-41
07.08.2010	15:33	47	150	160	0,060	5	0,060	2,38	6,5	-136
08.08.2010	15:45	71	150	160	0,079	4	0,079	3,40	6,6	-97
09.08.2010	16:16	95	150	160	0,067	1	0,067	0,07	6,7	-219
10.08.2010	11:14	114	150	160	0,073	5	0,073	1,37	6,6	-201
11.08.2010	13:29	140	150	160	0,069	7	0,069	0,48	6,6	-361
12.08.2010	11:34	163	150	160	0,080	8	0,080	2,35	6,2	-337
13.08.2010	16:25	191	150	160	0,070	10	0,070	0,70	6,1	-340

14.08.2010	16:29	215	150	160	0,093	15	0,093	5,10	6,9	-367
15.08.2010	17:36	241	150	160	0,173	20	0,173	16,31	6,8	-342
16.08.2010	19:17	266	150	160	0,243	15	0,243	14,85	6,7	-314
17.08.2010	16:19	287	150	160	0,281	15	0,281	10,30		
18.08.2010	16:19	311	150	160	0,336	15	0,336	13,84		
19.08.2010	16:20	335	150	160	0,316	10	0,316	3,16		
20.08.2010	16:31	360	150	160	0,289	10	0,289	2,89		
21.08.2010	16:31	384	150	160	0,281	10	0,281	2,81		
22.08.2010	16:14	407	150	160	0,240	10	0,240	2,40		
23.08.2010	16:57	432	150	160	0,179	0	0,179	0,00		
24.08.2010	13:21	452	150	160	0,161	0	0,161	0,00		
25.08.2010	16:34	480	150	160	0,172	10	0,172	3,48		
26.08.2010	14:00	501	150	160	0,211	15	0,211	9,41		
27.08.2010	14:53	526	150	160	0,273	15	0,273	14,02		
28.08.2010	16:14	551	150	160	0,273	17	0,273	4,64		
29.08.2010	17:30	577	150	160	0,243	10	0,243	2,43		
30.08.2010	19:54	602	150	160	0,187	0	0,187	0,00		
31.08.2010	16:01	627	150	160	0,148	10	0,148	1,48		
01.09.2010	18:01	653	150	160	0,117	15	0,117	1,76		
02.09.2010	17:04	678	150	160	0,083	5	0,083	0,41		
03.09.2010	16:50	703	150	160	0,055	12	0,055	0,66		
04.09.2010	20:35	729	150	160	0,033	10	0,033	0,33		
05.09.2010	16:42	754	150	160	0,022	10	0,022	0,22		
06.09.2010	16:09	779	150	160	0,007	0	0,007	0,00		
07.09.2010	11:21	805	150	160	0,006	0	0,006	0,00		

08.09.2010	13:54	830	150	160	0,009	0	0,009	0,53		
09.09.2010	18:05	855	150	160	0,009	0	0,009	0,00		
10.09.2010	09:34	881	150	160	0,013	0,5	0,013	0,66		

Table A.28 Raw data for HRT = 6 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vtotal gas (ml)***	***H_2.1 (%)***	***H_2 Production (ml/day)***	***pH***	***ORP***
				310	0	0	0	0		
05.08.2010	17:00	0	150	160	0,000	0	0,000	0,00	7,00	
06.08.2010	18:10	25	150	160	0,080	6	0,080	13,28	6,1	-41
07.08.2010	15:33	47	150	160	0,101	3	0,101	3,66	6,5	-136
08.08.2010	15:45	71	150	160	0,096	0	0,096	0,00	6,6	-97
09.08.2010	16:16	95	150	160	0,076	0	0,076	0,00	6,7	-219
10.08.2010	11:14	114	150	160	0,064	0	0,064	0,00	6,6	-201
11.08.2010	13:29	140	150	160	0,052	0	0,052	0,00	6,6	-361
12.08.2010	11:34	163	150	160	0,047	12	0,047	0,56	6,2	-337
13.08.2010	16:25	191	150	160	0,057	20	0,057	2,67	6,1	-340
14.08.2010	16:29	215	150	160	0,069	10	0,069	2,61	6,9	-367
15.08.2010	17:36	241	150	160	0,094	0	0,094	4,11	6,8	-342
16.08.2010	19:17	266	150	160	0,132	35	0,132	10,65	6,7	-314
17.08.2010	16:19	287	150	160	0,166	45	0,166	12,91		
18.08.2010	16:19	311	150	160	0,232	45	0,232	21,00		
19.08.2010	16:20	335	150	160	0,260	60	0,260	20,08		

20.08.2010	16:31	360	150	160	0,229	30	0,229	6,87		
21.08.2010	16:31	384	150	160	0,275	15	0,275	11,49		
22.08.2010	16:14	407	150	160	0,313	10	0,313	9,21		
23.08.2010	16:57	432	150	160	0,344	0	0,344	4,96		
24.08.2010	13:21	452	150	160	0,463	0	0,463	19,04		
25.08.2010	16:34	480	150	160	0,524	40	0,524	30,72		
26.08.2010	14:00	501	150	160	0,633	25	0,633	33,27		
27.08.2010	14:53	526	150	160	0,690	40	0,690	36,72		
28.08.2010	16:14	551	150	160	0,738	22	0,738	23,92		
29.08.2010	17:30	577	150	160	0,665	30	0,665	19,95		
30.08.2010	19:54	602	150	160	0,682	10	0,682	9,54		
31.08.2010	16:04	627	150	160	0,609	18	0,609	10,96		
01.09.2010	18:03	653	150	160	0,552	10	0,552	5,52		
02.09.2010	17:07	678	150	160	0,482	15	0,482	7,23		
03.09.2010	16:52	703	150	160	0,368	5	0,368	1,84		
04.09.2010	20:37	729	150	160	0,303	0	0,303	0,00		
05.09.2010	16:45	754	150	160	0,262	5	0,262	1,31		
06.09.2010	16:11	779	150	160	0,158	0	0,158	0,00		
07.09.2010	11:23	805	150	160	0,140	0	0,140	0,00		
08.09.2010	13:57	830	150	160	0,115	0	0,115	0,00		
09.09.2010	18:07	855	150	160	0,093	0	0,093	0,00		
10.09.2010	09:36	881	150	160	0,084	0	0,084	0,00		
11.09.2010	18:06	906	150	160	0,081	0	0,081	0,00		
12.09.2010	17:09	931	150	160	0,056	0	0,056	0,00		
13.09.2010	17:48	957	150	160	0,041	0	0,041	0,00		

14.09.2010	18:42	982	150	160	0,035	0	0,035	0,00		
15.09.2010	16:51	1008	150	160	0,049	0	0,049	2,18		
16.09.2010	16:49	1033	150	160	0,054	2,5	0,054	0,92		
17.09.2010	16:55	1058	150	160	0,077	2	0,077	3,80		
18.09.2010	19:40	1084	150	160	0,094	2	0,094	2,97		
19.09.2010	16:54	1109	150	160	0,105	0	0,105	1,76		

Table A.29 Raw data for HRT = 8 days

DATE	***HOUR***	***TIME (h)***	***Vs (ml)***	***Vg (ml)***	***H_2.1 (%)***	***Vtotal gas (ml)***	***H_2.1 (%)***	***H_2 Production (ml/day)***	***pH***	***ORP***
				310	0	0	0	0		
05.08.2010	17:00	0	150	160	0,000	0	0,000	0,00	7,00	
06.08.2010	18:10	25	150	160	0,103	12	0,103	17,72	6,1	-41
07.08.2010	15:33	47	150	160	0,121	0	0,121	2,88	6,5	-136
08.08.2010	15:45	71	150	160	0,108	0	0,108	0,00	6,6	-97
09.08.2010	16:16	95	150	160	0,082	0	0,082	0,00	6,7	-219
10.08.2010	11:14	114	150	160	0,070	0	0,070	0,00	6,6	-201
11.08.2010	13:29	140	150	160	0,056	0	0,056	0,00	6,6	-361
12.08.2010	11:34	163	150	160	0,054	0	0,054	0,00	6,2	-337
13.08.2010	16:25	191	150	160	0,044	15	0,044	0,66	6,1	-340
14.08.2010	16:29	215	150	160	0,041	20	0,041	0,82	6,9	-367
15.08.2010	17:36	241	150	160	0,039	0	0,039	0,00	6,8	-342
16.08.2010	19:17	266	150	160	0,042	25	0,042	1,49	6,7	-314

17.08.2010	16:19	287	150	160	0,066	37	0,066	6,32		
18.08.2010	16:19	311	150	160	0,186	40	0,186	26,67		
19.08.2010	16:20	335	150	160	0,272	70	0,272	32,80		
20.08.2010	16:31	360	150	160	0,413	40	0,413	39,08		
21.08.2010	16:31	384	150	160	0,534	40	0,534	40,72		
22.08.2010	16:14	407	150	160	0,570	30	0,570	22,86		
23.08.2010	16:57	432	150	160	0,602	0	0,602	5,12		
24.08.2010	13:21	452	150	160	0,642	0	0,642	6,40		
25.08.2010	16:34	480	150	160	0,560	0	0,560	0,00		
26.08.2010	14:00	501	150	160	0,482	0	0,482	0,00		
27.08.2010	14:53	526	150	160	0,488	0	0,488	0,96		
28.08.2010	16:14	551	150	160	0,462	0	0,462	0,00		
29.08.2010	17:30	577	150	160	0,474	0	0,474	1,92		
30.08.2010	19:54	602	150	160	0,487	0	0,487	2,08		
31.08.2010	16:07	627	150	160	0,457	7	0,457	3,20		
01.09.2010	18:07	653	150	160	0,440	0	0,440	0,00		
02.09.2010	17:10	678	150	160	0,393	0	0,393	0,00		
03.09.2010	16:52	703	150	160	0,342	0	0,342	0,00		
04.09.2010	20:39	729	150	160	0,247	0	0,247	0,00		
05.09.2010	16:47	754	150	160	0,199	0	0,199	0,00		
06.09.2010	16:14	779	150	160	0,128	0	0,128	0,00		
07.09.2010	11:25	805	150	160	0,120	0	0,120	0,00		
08.09.2010	13:59	830	150	160	0,102	0	0,102	0,00		
09.09.2010	18:10	855	150	160	0,094	0	0,094	0,00		
10.09.2010	09:39	881	150	160	0,087	0	0,087	0,00		

11.09.2010	18:10	906	150	160	0,080	0	0,080	0,00		
12.09.2010	17:11	931	150	160	0,061	0	0,061	0,00		
13.09.2010	17:50	957	150	160	0,055	0	0,546	0,00		
14.09.2010	18:45	982	150	160	0,049	0	0,049	0,00		
15.09.2010	16:53	1008	150	160	0,040	0	0,040	0,00		
16.09.2010	16:51	1033	150	160	0,029	0	0,029	0,00		
17.09.2010	16:57	1058	150	160	0,023	0	0,023	0,00		
18.09.2010	19:43	1084	150	160	0,027	0	0,027	0,59		
19.09.2010	16:56	1109	150	160	0,041	0	0,041	2,35		

B.1 Nomenclature

A	Axial point in Box-Wilson statistical experiment design
$C_{H2,i}$	H_2 content in the head space of the i^{th} measurement, %
$C_{H_2,i-1}$	H_2 content in the head space of the $i\text{-}1^{th}$ measurement, %
CHF	Cumulative hydrogen formation, mL
ΔGo	Gibbs free energy, kJ
H	Cumulative hydrogen at any time, mL
ΔH	Produced cumulative hydrogen, moles or mL
HPR	Hydrogen production rate, $mLH_2\ h^{-1}$
HRT	Hydraulic residence time, h
HY	Hydrogen yield, mol H_2 mol glucose^{-1}
I	Light intensity, lux
K	Temperature, Kelvin
Ks	Saturation constant, gL^{-1}
K_I	Substrate inhibition constant, gL^{-1}
k	Specific hydrogen production rate constant, $mLH_2\ g^{-1}$biomass h^{-1}
λ	Lag phase, hour
μmax	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	Flow rate, 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}
So	Initial total sugar concentration, gL^{-1}
Sf	Final total sugar 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 i^{th} measurement, mL
$V_{G_{i-1}}$	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

AHWS	Acid hydrolysed wheat starch
ANS	Anaerobic sludge
ATP	Adenosin triphosphate
C/N	Carbon to nitrogen ratio
C/P	Carbon to phosphorous ratio
CSTR	Completely stirred reactor
DFE	Dark fermentation effluent
EDTA	Ethylenediaminetetraacetic acid
GC	Gas choromatograph
HPLC	High performance liquid chromatograph
HAc	Acetic acid
HBu	Butyric acid
HPr	Propionic acid
HLa	Lactic acid
IR	Infrared lamp
NRLL	US National Centre for Agricultural Utilization Research
PNS	Purpule non-sulphur bacteria
TVFA	Total volatile fatty acid
TS	Total sugar
VFA	Volatile fatty acid
WW	wastewater
WP	Wheat powder