DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOHYDROGEN PRODUCTION FROM HYDROLIZED WASTE WHEAT BY CONTINUOUS DARK FERMENTATION PROCESS CONTAINING NOVEL SUPPORT MATERIAL

by
Firuze KARAOSMANOĞLU

January, 2015
İZMİR

BIOHYDROGEN PRODUCTION FROM HYDROLIZED WASTE WHEAT BY CONTINUOUS DARK FERMENTATION PROCESS CONTAINING NOVEL SUPPORT MATERIAL

A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of
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Degree of Master of Science in Environmental Engineering Program

by
Firuze KARAOSMANOĞLU

January, 2015
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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"BIOHYDROGEN PRODUCTION FROM HYDROLIZED WASTE WHEAT BY CONTINUOUS DARK FERMENTATION PROCESS CONTAINING NOVEL SUPPORT MATERIAL"** completed by **FİRUZE KARAOSMANOĞLU** under supervision of **PROF. DR. İLGİ KARAPINAR KAPDAN** 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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Firuze KARAOSMANOĞLU

BIOHYDROGEN PRODUCTION FROM HYDROLIZED WASTE WHEAT BY CONTINUOUS DARK FERMENTATION PROCESS CONTAINING NOVEL SUPPORT MATERIAL

ABSTRACT

The aim of this thesis is biohydrogen production from waste wheat in immobilized systems by continuous dark fermentation using up-flow packed bed reactor. For this purpose, acid hydrolysed wheat powder was used as substrate. Waste wheat was sieved down to 70 micrometer size (less than 200 mesh) and acid hydrolysed at pH 2 and 90 degrees Celcius in an autoclave for 15 minutes. The resulting sugar solution was used for biohydrogen production by dark fermentation after neutralization and nutrient addition. The microbial growth support particle was aquarium biological sponge. Heat-treated anaerobic sludge was used as inoculum. Total gas volume and hydrogen percentage in total gas, hydrogen gas volume, initial and final total sugar and total volatile fatty acids concentration were monitored daily throughout the experiments. Effects of hydraulic retention time and initial substrate concentration on the rate and yield of hydrogen gas formations were investigated. In order to determine the optimal hydraulic retention time yielding the highest hydrogen formation, the reactor was operated at between 1 and 8 hour by keeping initial substrate concentration constant at 15 gram per liter. Daily hydrogen production yield and rate reached the highest level at hydraulic retention time of 3 hour. Effects of initial substrate concentration on dark fermentation performance was investigated by varying initial sugar concentration between 10 gram per liter and 35 gram per liter at hydraulic retention time is 3 hour. Hydrogen production volume and rate were maximum at sugar concentration of 25 gram per liter. The yield reached to its maximum value at 19 gram per liter. Both the hydraulic retention time and initial sugar concentration had considerable effects on biohydrogen production from hydrolysed waste wheat by continuous dark fermentation.

Keywords: Biohydrogen, wheat powder, dark fermentation, anaerobic sludge, immobilization, up-flow packed bed reactor, continuous operation.

HİDROLİZE ATIK BUĞDAYDAN YENİ BİR DESTEK TANECİĞİ KULLANILARAK SÜREKLİ İŞLETİLEN KARANLIK FERMANTASYON SÜRECİ İLE BİYOHİDROJEN ÜRETİMİ

ÖZ

Bu tezin amacı, yukarı akışlı dolgulu kolon reaktör kullanılarak sürekli beslemeli bağlı büyümeli biyolojik sistemlerde karanlık fermantasyon atık buğdaydan biyohidrojen üretimidir. Bu amaçla, substrat olarak asitle hidrolize edilmiş buğday tozu kullanılmıştır. Atık buğday 70 mikronmetre boyutunda (<200 mesh) elekten geçirilmiş ve pH=2'de, 15 dakika boyunca 90 santigrat derecede otoklavlanarak hidrolize edilmiştir. Elde edilen şeker çözeltisi nötralize edildikten ve nutrient ilavesi yapıldıktan sonra substrat olarak kullanılmıştır. Mikroorganizma destek taneciği olarak biyolojik akvaryum süngeri, aşı kültürü olarak ise asitle ön işlem görmüş anaerobik çamur kullanılmıştır. Deneylerde, günlük toplam gaz hacmi ve hidrojen yüzdesi, giriş ve çıkış şeker, toplam uçucu yağ asidi derişimleri ölçülmüştür. Çalışmada, hidrolik alıkonma süresinin ve başlangıç substrat derişiminin hidrojen gazı hacmi, hızı ve verimine olan etkileri incelenmiştir. En yüksek hidrojen üretimi sağlayan hidrolik alıkonma süresinin tespiti için sistem, 1 litrede 15 gram toplam şeker derişiminde ve 1 saat ile 8 saat arasında değişen hidrolik alıkonma sürelerinde işletilmiştir. Maksimum hidrojen üretim verimi ve hızı hidrolik alıkonma süresi 3 saat iken elde edilmiştir. Başlangıç substrat derişiminin etkisini incelemek amacıyla sistem, hidrolik alıkonma süresi 3 saatte ve 1 litrede 10 gram ve 35 gram başlangıç şeker derişimlerinde işletilmiştir. Hidrojen üretim hacmi ve hızı 1 litrede 25 gram başlangıç substrat derişiminde elde edilirken, üretim verimi maksimum değerine 1 litrede 19 gram başlangıç substrat derişimde ulaşmıştır. Hidrolik alıkonma süresi ve giriş şeker derişiminin biyohidrojen üretimi etkileyen iki önemli işletim parametresidir. En yüksek hidrojen üretim verimi için optimum işletim şartları 1 litrede 19 gram ve 3 saat hidrolik alıkonma süresi olarak belirlenmiştir.

Anahtar kelimeler: Biyohidrojen, buğday tozu, karanlık fermentasyon, anaerobik çamur, immobilizasyon, yukarı akışlı dolgulu kolon reaktör, sürekli işletim.

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CHAPTER ONE
INTRODUCTION

1.1 Energy Problems

Energy is a vital factor of material comfort and a source of many of the largest effects of human beings on their environment. Although energy sources are crucial for the world's future, they are decreasing from day to day. If energy is insufficient or too expensive, people will face lack of direct energy services such as lighting, cooking, heating, transport and some economic problems (inflation, unemployment etc.). For many years, fossil originating fuel reserves, which are present in nature in extensive amount, were used as an energy source. In the last decades, scientists determined that the current energy systems lead to disastrous effects such as air pollution. Burning fossil fuels releases carbon dioxide, nitrogen monoxide, nitrogen dioxide, sulphur dioxide, carbon monoxide etc. These gases called as greenhouse gases and they affect human health, cause air pollution and they are the main aspect of global warming. As a consequence, scientist trying to find alternative energy sources that could be replaced by fossil fuels. Hydrogen energy is an important source of clean energy plan and it is a good candidate to be a main energy source.

1.2 The Properties of Hydrogen

Hydrogen was discovered in the 1500' s and in 1700' s was awared as flammable. It is a non-toxic, the simplest and lightest element and 14.4 times lighter than air. It is also the most plentiful element in the universe (Momirlan & Veziroğlu, 2005). Hydrogen content of the atmosphere and Earth's surface is 0.07 % and 0.14 %, respectively. The density of hydrogen (0.09 g/ L) is quite small as compared with the density of air (1.2 g/ L) (Argun, 2010). It can not be exist alone in nature, it is found in water body and so many organic and chemical compounds. It has no colour and odor. It can be transform to liquid form at -252.77 °C. The heating value of hydrogen is 3042 cals/ m^3 (considering water as a product). In combustion, water is the main product, thus, hydrogen is regarded as a clean non-polluting fuel. The properties of different fuels are summarized in Table 1.1. As compared to other gaseous fuels, hydrogen is harmless to humans and the environment (Das & Veziroğlu, 2001).

Table 1.1 Fuel properties at 25°C and 1 atm (White, Steeper, & Lutz, 2006).

Property	Hydrogen	Compressed Natural Gas	Gasoline
Density (kg/ m^3)	0.0824	0.72	730[a]
Flammability limits (volume % in air)	4-78	4.3-15	1.4-7.6
Flammability limits (ϕ)	0.1-7.1	0.4-1.6	≈0.7-4
Autoignition temperature in air (K)	858	723	550
Minimum ignition energy (mJ)[b]	0.02	0.28	0.24
Flame velocity (m s^{-1})[b]	1.85	0.38	0.37-0.43
Adiabatic flame temperature (K)[b]	2480	2214	2580
Quenching distance (mm)[b]	0.64	2.1[c]	≈2
Stoichiometric fuel/air mass ratio	0.029	0.069	0.068
Stoichiometric volume fraction %	29.53	9.48	≈2[d]
Lower heating value (MJ/kg)	119.7	45.8	44.79
Heat of combustion (MJ/kg_{air})[b]	3.37	2.9	2.83

[a] Liquid at 0 °C.
[b] At stoichiometry.
[c] Methane.
[d] Vapor.

1.3 Uses and Advantages of Hydrogen Gas

Hydrogen energy is a key factor of new sustainable clean energy systems. It is economic and safe. Uses and applications of hydrogen gas are as follows:

- Reactant of hydrogenation processes.
- Used for methanol production.
- Chemical reagent that protects from oxidation and corosion.
- Metal sintering and annealing processes.
- In aerospace and aircraft industry as a power source.
- Cooler of electric generator.

- Automotive and transportation equipment as an alternative fuel.
- For welding stainless steel with mixing argon gas.
- Hydrogen gas also used in chemical, electronic, food and bevarage, glass, metal production and pharmaceutical and biotechnology industries.

This new energy carrier has some important advantages:

- Transportation of hydrogen gas is safe.
- There are so many production methods of hydrogen (e.g. fossil fuels, electrolyses of water, directly by sunlight etc.)
- Hydrogen can be used advantageously as a chemical feedstock in the petrochemical, food, microelectronics, ferrous and non-ferrous metal, chemical and polymer synthesis, and metallurgical process industries, and as an energy carrier in clean sustainable energy systems (Midilli, Ay, Dinçer, & Rosen, 2005).
- The combustion of hydrogen does not produce CO_2, CO, SO_2, VOC and particles, but entails emission of vapour and NO_X. The formation of NOx is a function of flame temperature and duration. Considering the wide flammability range of hydrogen, its combustion can be influenced by the design of the engine so that the NOx emission can be reduced (Momirlan & Veziroğlu, 2002).

1.4 Conventional Hydrogen Production Methods

Hydrogen has great potential as an alternative fuel and hence considered as clean energy carrier. There are various technologies for the production of hydrogen. Basically, hydrogen production methods divided into 3 parts: producion from fossil fuels, biomass and water. Distribution of hydrogen gas production from coal, electrolysis, natural gas and oil is shown in Figure 1.1. Table 1.2 summarizes the basic hydrogen production processes, raw material source, energy need and air emissions.

The methods of hydrogen production from fossil fuels are (Das & Veziroğlu, 2001):

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

Methods of hydrogen production from biomass are;

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

Methods of hydrogen production from water are;

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

CO_2 is one of the greenhouse gases and most of the conventional hydrogen production processes cause CO_2 emission. A comparison for hydrogen production processes from different fuel sources and their carbon dioxide emission profile is given in Table 1.3.

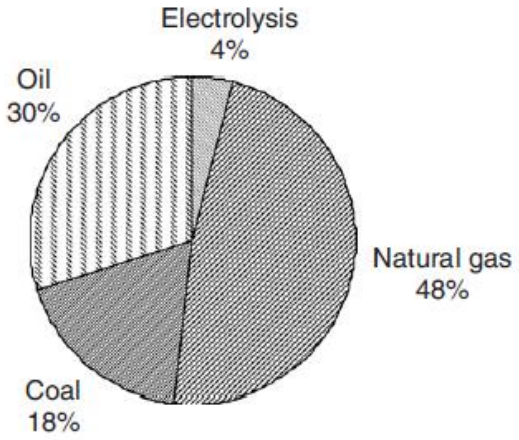


Figure 1.1 Feedstock used in the current global production of hydrogen (Kothari, Buddhi & Sawhney, 2008).

Table 1.2 Basic hydrogen production processes, raw material source, energy need and air emissions (Aksöyek, 2011).

Hydrogen Production Method	Process	Source	Energy	Emission
Thermal	Steam Reforming	Natural Gas	Steam at high temperature	CO_2, CO
	Thermochemical Water Splitting	Water	High temperature comes from nuclear reaktor	No Emission
	Gasification	Coal & Biomass	Water vapor and oxygen at high temperature and pressure	CO_2, CO
	Pyrolysis	Biomass	Water vapor at mild temperature	CO_2, CO
Electro chemical	Electrolysis	Water	Electiricity (wind, sun, nuclear)	No emission
	Electrolysis	Water	Electiricity (coal and natural gas)	Emission arise from electricity production
	Photo chemical	Water	Sun	No emission
Biological	Photo biological	Algaes	Sun	No emission
	Anaerobic fermantation	Biomass	High temperature	Slight quantity
	Fermentative microorganisms	Biomass	High temperature	Slight quantity

Table 1.3 Hydrogen production processes from different fuel sources and their carbon dioxide emission profile (Kothari et al., 2008).

Process	Reactions	H_2 from H_2O (%)	CO_2/ H_2 (Nm^3/ Nm^3)
Steam reforming+CO conversion	$C_nH_m + nH_2O \rightarrow nCO + (n+m/2)\ H_2$		
	$_nCO + nH_2O \rightarrow nCO_2 + nH_2$		
From			
Methane	$CH_4 + 2H_2O \rightarrow CO_2 + 4H_2$	50.0	0.25
Ethane	$C_2H_6 + 4H_2O \rightarrow 2CO_2 + 7H_2$	57.1	0.29
Pentane	$C_5H_{12} + 10H_2O \rightarrow 5CO_2 + 16H_2$	62.5	0.31
Naphta	$C_{10}H_{22} + 20H_2O \rightarrow 10CO_2 + 31H_2$	64.51	0.32
Partial oxidation of hydrocarbons (heavier than naphta)	$2C_nH_m + H_2O + 23/2O_2 \rightarrow nCO + nCO_2 + (m+1)H_2$		
From	$2C_8H_{18} + H_2O + 23/2O_2 \rightarrow 8CO + 8CO_2 + 19H_2$	5.3	0.42
Coal gasification	$CH_{0.8} + 0.6H_2O + 0.7O_2 \rightarrow CO_2 + H_2$	70	1.00
Electrolysis (PV, Wind, hydro)	$2H_2O \rightarrow 2H_2 + O_2$	100	0

In summary, hydrogen production methods from fossil fuels as thermochemical and electrochemical prosesses are not clean energy source because of their gas emissions and intensive energy requirement. On the other hand, biological hydrogen production processes are mostly operated at ambient temperatures and pressures, thus less energy intensive. These processes are not only environment friendly, but also lead to open a new avenue for the utilization of renewable energy resources which in exhaustable (Das & Veziroğlu, 2001).

1.5 Biological Hydrogen Production Methods

Biohydrogen production has been established as a prospective alternative and integral component of green sustainable energy (Das & Veziroğlu, 2001). They can be classified into 4 approaches as (i) bio-photolysis of water by algae (ii) photo-fermentation (iii) dark-fermentation (iv) combination of photo-fermentation and dark-fermentation (Das & Veziroglu, 2001; Kapdan & Kargı, 2006). The schemetic

diagram of these approches is given in Figure 1.2 and comparison of all processes is depicted in Figure 1.3.

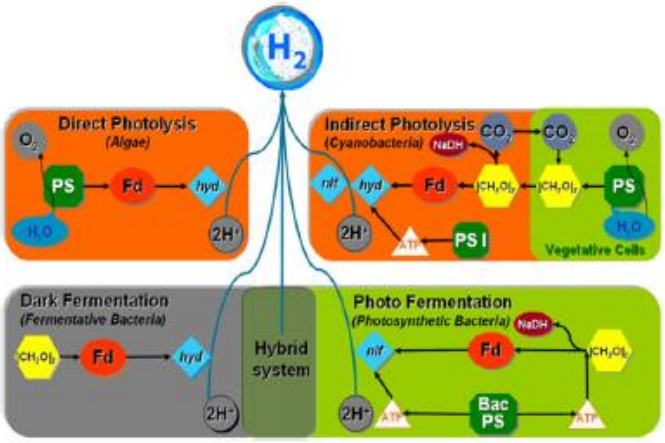


Figure 1.2 Biohydrogen production methods (Kotay & Das, 2008).

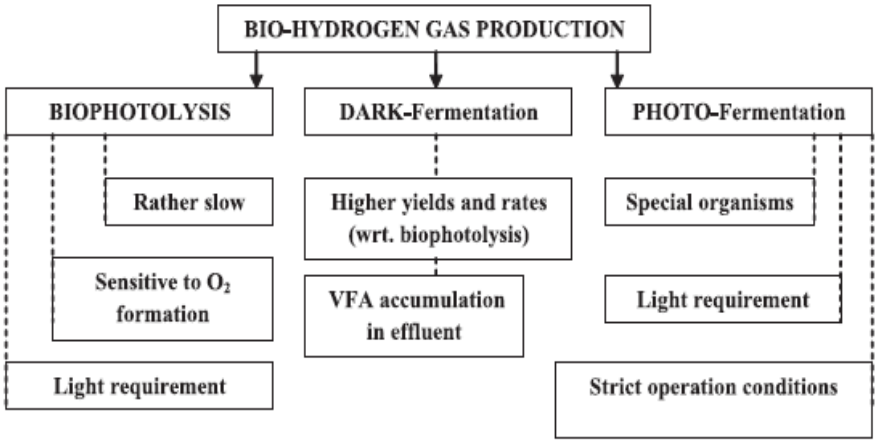


Figure 1.3 Comparison of biohydrogen production by algae and bacterial processes (Argun & Kargı, 2011).

All processes are controlled by the hydrogen-producing enzymes, such as hydrogenase and nitrogenase. The major components of nitrogenase are MoFe protein and Fe protein. Nitrogenase has the ability to use magnesium adenosine triphosphate (MgATP) and electrons to reduce a variety of substrates including protons. This chemical reaction yields hydrogen by a nitrogenase-based system as follows (Hallenbeck & Benemann, 2002):

$$2e^- + 2H^+ + 4ATP \rightarrow H_2 + 4ADP + 4Pi \qquad (1.\ 1)$$

Hydrogenases exist in most of the photosynthetic microorganisms and they can be classified into two categories:

- Uptake hydrogenases: Uptake hydrogenases, such as NiFe hydrogenases and NiFeSe hydrogenases, act as important catalysts for hydrogen consumption as follows:

$$H_2 \rightarrow 2e^- + 2H^+ \quad (1.2)$$

- Reversible hydrogenases: Reversible hydrogenases, as indicated by its name, have the ability to produce H_2 as well as consume H_2 depending on the reaction condition,

$$H_2 \leftrightarrow 2e^- + 2H^+ \quad (1.3)$$

Properties of hydrogenase and nitrogenase systems are compared in Table 1.4

Table 1.4 Comparison of hydrogenase and nitrogenesae enzymes for hydrogen production (Lin, Harada, Suzuki & Hatano, 2005).

Property	Nitrogenase	Hydrogenase
Substrates	ATP, H^+ or N_2, electrons	H^+, H_2
Products	H_2 or NH_4^+	ATP, H^+,H_2, electrons
Number of proteins	Two (Mo-Fe and Fe)	One
Metal components	Mo, Fe	Ni, Fe, S
Optimal temperature	30 °C *(A.vinelandii)*	55 °C*(R.rubrum),* 70 °C*(R.capsulatus)*
Optimal pH	7.1-7.3 *(A.vinelandii)*	6.5- 7.5 *(R.sulfidophilus)*
Inhibitors	N_2 (of H_2 production), NH_4^+, O_2, high N to C ratio	CO, EDTA (ethlenediaminetetraacedic acid), O_2, presence of organic compound
Simulators	Light	H_2 (*R.sphaeroides*), absence of organic compounds (*R.rubrum, R.capsulatus)*

1.5.1 Biophotolysis of Water by Algae

Hydrogen production from biophotolysis of water is achieved by using green algae and blue - green algae (cyanobacteria) (Das & Veziroglu, 2008). Biophotolysis by algae divided into 2 parts: direct biophotolysis and indirect biophotolysis. Algae split water molecules to hydrogen and oxygen using light energy. The generated hydrogen ions are converted into hydrogen gas by hydrogenase enzyme as given in reaction 4 (Kapdan & Kargı, 2006) and Figure 1.4.

$$2H_2O + \text{lightenergy} \rightarrow 2\ H_2 + O_2 \qquad (1.4)$$

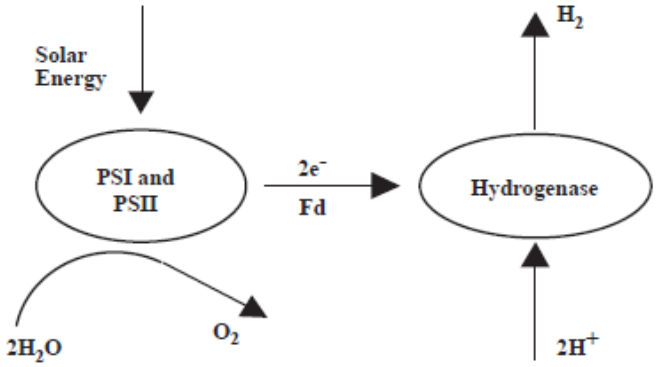


Figure 1.4 Schematics of direct biophotolysis (Ni,Leung Y.C., Leung K.H. & Sumathy, 2006).

Biohydrogen production from direct photolysis can produce hydrogen directly from water and sunlight. This system also provides tenfolds solar conversion energy increase in comparison to trees and crops. On the other hand, green algae require high intensity of light and this could be not possible always (Nath & Das, 2004). Beside to this, oxygen generated during hydrogen production could be inhibitory. Blue-green algae called as cyanobacteria take place in indirect biophotolysis. Like as direct biophotolysis, light energy is used. The reaction of indirect biopholysis of water and glucose by cyanobacteria are given equation 5 and equation 6, respectively (Das & Veziroglu, 2008). The schemetic diagram of indirect biopholoysis is depicted in Figure 1.5.

$$12H_2O + 2\ CO_2 + \text{lightenergy} \rightarrow C6H_{12}O_6 + 6O_2 \qquad (1.5)$$

$$C6H_{12}O_6 + 12H_2O + \text{lightenergy} \rightarrow +12\ H_2 + 6CO_2 \qquad (1.6)$$

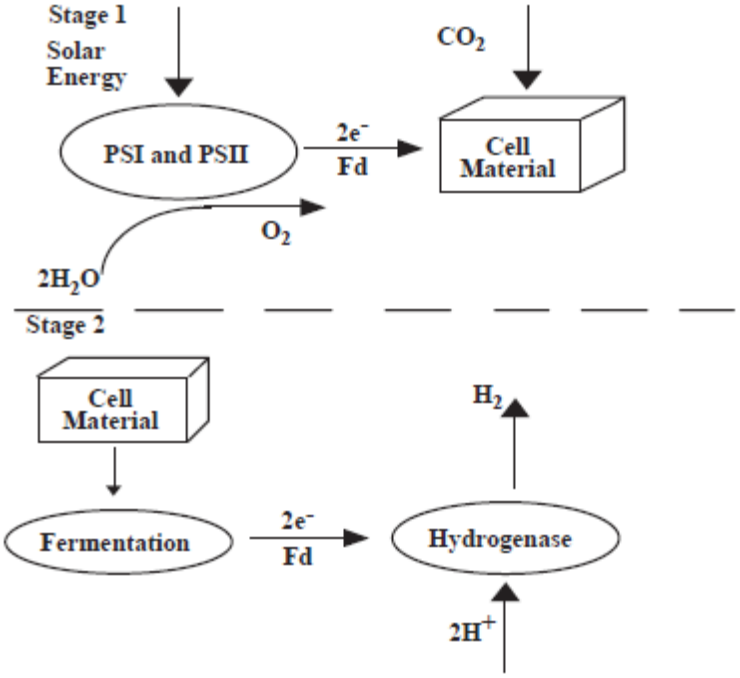


Figure 1.5 Schematics of indirect biophotolysis (Ni et al., 2005).

One of the advantage of hydrogen production by the indirect biophotolysis is utilization water as raw material. Furthermore, cyanobacteria has the ability to fix N_2 from atmosphere. But in the end of this hydrogen production process, photochemical efficieny is insufficient. Hydrogenase enzymes should be removed to stop degradation of hydrogen is another disadvantage of indirect bio-photolysis process (Nath & Das, 2004).

1.5.2 Photo Fermentation

Anaerobic and photosynthetic bacteria are the major agent of hydrogen production by photo-fermentation (Kapdan, Kargı, Oztekin & Argun, 2009). A wide spectral energy and different waste material can be used by these bacteria (Nath & Das, 2004). They produce hydrogen through the action of their nitrogenase enzyme in the presence of light by using organic acids or simple sugar as carbon source. Equation of hydrogen production from organic acids by the photo-fermentation is given in equation 7 (Kapdan et al., 2009) and the schemetic diagram of process is shown in Figure 1.6 (Ni et al., 2005).

$$2CH_3COOH + 4H_2O + \text{'light energy'} \rightarrow 8H_2O + 4CO_2 \qquad (1.\ 7)$$

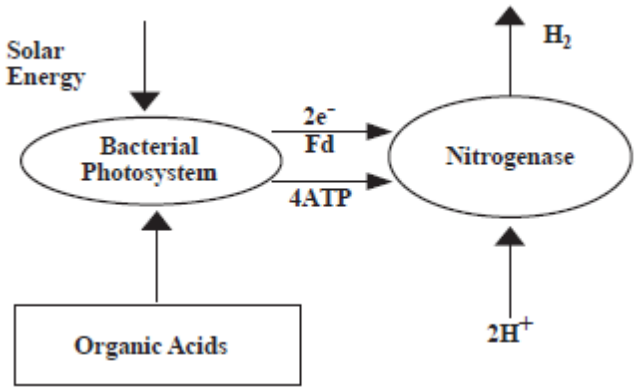


Figure 1.6 A scheme of photo-fermentation (Ni et al., 2005).

Several types of *Rhodobacter* species were used as photo fermentative bacteria for hydrogen gas production. *Rhodobacter* species convert into volatile fatty acids (VFAs) into hydrogen and carbon dioxide (Koku et al., 2003; Kapdan, Kargı, Oztekin & Argun, 2009). Photo fermentative bacteria need complex nutrients, such as EDTA, Mo and Fe, strict control of temperature, pH, light intensity and non-inhibitory concentrations of VFAs and NH4-N. These enviromental conditions have to be 30-35 °C, 6.8-7.5, 4-10 klux, <2500 mg/ L and < 50 mg/ L, respectively (Argun & Kargı, 2010). There are several disadvantages of photofermentative hydrogen production. One of the drawbacks is the low solar energy conversion efficiencies. Only 1-5 % light conversion efficiency can be achieved. Another one is the nitrogenase enzyme with its inherent high energy demand. Moreover, oxygen is a strong inhibitor of hydrogenase enzyme (Hallenbeck & Benemann, 2002; Nath & Das, 2004). Anaerobic photobioreactors covering large areas, additionally (Fedorov, Tsygankov, Rao & Hall, 1998). These drawbacks make the photofermentative hydrogen production from organic materials a secondary alternative. The process need detailed scientific studies in order to overcome all these problems and also improve the process.

1.5.3 Dark Fermentation

The fermentative conversion of organic substrates to biohydrogen under anaerobic conditions is defined as dark-fermentation. The main catalyst of dark-fermentation is hydrogenase enzyme. Carbon is provided from mainly monosaccharides which can be generated by acid or enzymatic hydrolysis of polysaccharides such as starch or cellulose (Vazques & Varaldo, 2009; Argun, Kargı, Kapdan & Oztekin, 2008; Cai, Jin, Saint & Monis, 2010; Sagnak, Kapdan, Kargı; 2010). Biohydrogen production from dark-fermentation is carried out by the anaerobic metabolism of pyruvate, formed during the catabolism of various substrates. Equation 7 and 8 shows the breakdown of pyruvate (Hallenbeck & Benemann, 2002).

$$\text{Pyruvate} + \text{CoA} + 2\text{Fd(oxidized)} \rightarrow \text{Acetil-CoA} + 2\text{Fd(reduced)} + CO_2 \quad (1.8)$$

$$2\text{Fd(reduced)} \rightarrow 2\text{Fd (oxidized)} + H_2 \quad (1.9)$$

The schemetic pathway of glucose conversion to hydrogen via fermentation is given in Figure 1.7 (Vazquez & Varaldo, 2009). The glucose is catabolized to pvruvate first and then oxizided to acetyl-CoA. In the following step, acetly-CoA is converted into acetly phospate. Acetly phosphate provides the generation of ATP and the release of acetate. Pyruvate oxidation to acetyl-CoA requires ferredoxin (Fd) reduction. Reduced Fd is oxidized by hydrogenase which generates Fd and releases electrons as molecular hydrogen (Nath & Das, 2004).

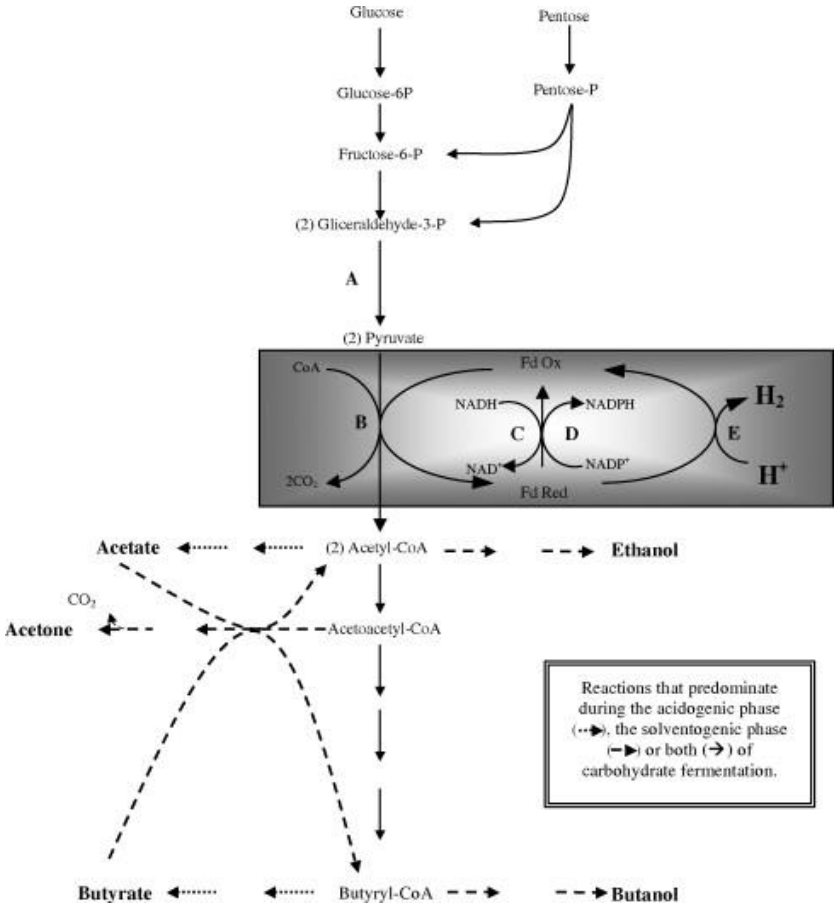


Figure 1.7 Systematic diagram of carbohydrate fermentation to H_2, CO_2, organic acids and solvents during dark fermentation by *Clostridial* sp (Vazquez & Varaldo, 2009).

Theoretical maximum hydrogen yield of dark fermentation is 4 mol H_2 mol/ glucose if acetic acid is the only VFA formed. (Argun, Kargı & Kapdan, 2009). The stechiometric equation of glucose conversion to acetic acid through which hydrogen generation is given in equation 10 (Manish & Banerjee, 2008). Since the free energy change is negative, bacteria can complete the conversion without any external energy supply (Argun, 2010).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4\ H_2 + 2CO_2 + 2CH_3COOH \quad \Delta G_0 = -206\ kJ \quad (1.\ 10)$$

If end product is only butyric acid, 2 mol H_2 per mole of glucose is obtained as shown in equation 11. Generation of propionic acid consumes 1 mol of H_2 per mole of propionic acid formed as presented in equation 12 (Argun & Kargi, 2011).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \Delta G_0 = -206\ kJ \quad (1.\ 11)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_2CH_2CH_2COOH + 2CO_2 + 2H_2 \quad \Delta G_0 = -206\ kJ \quad (1.12)$$

Dark fermentative bacteria can produce hydrogen without light energy and a variety of carbon sources can be used as a substrate. By products of dark fermentation are valuable metabolities such as lactic and acetic acids. Dark fermentation is the most efficient and economic method of biohydrogen production (Nath & Das, 2004). Dark fermentation usually achieves a much higher hydrogen production yield than other biological processes and this method provide waste reduction and hydrogen generation (Lee, Lin & Chang, 2006).

1.5.4 Combination of Photo Fermentation and Dark Fermentation

Combination of dark and light anaerobic fermentation is used for biohydrogen production from carbohydrate rich biomass or waste material (Kapdan & Kargı, 2006). Steps of biohydrogen production by dark and light fermentation of glucose is shown in Figure 1.8.

Acid or enzymatic hydrolysis of biomass to sugar solution is the first step of biohydrogen generation. Acid hydrolysis can be achieved in dilute acid solution at high temperature and at 1 atm pressures. This hydrolysis method is simple and fast. But require energy, high chemical consumption and neutralization which means additional chemical cost. Enzymatic hydrolysis of biomass or complex carbohydrates is advantageous over acid hydrolysis in terms of no chemical consumption and pure end product formation which is glucose. However, the rate of hydrolysis by enzyme is low and cost of enzyme is high. Therefore, acid pretreatment is still more preffered in the hydrolysis of biomass. As glucose or sugar solution is obtained after hydrolysis, the next stage is dark fermentation of this sugar solution for simultaneous hydrogen generation and organic acid formation. The end product of dark fermention is subjected to photofermentation to get further hydrogen (Kapdan & Kargı, 2006; Kotay & Das, 2008).

Dark fermentation with mainly acidogenic bacteria (*Clostridium* sp. and *Enterobacter* sp.) could produce hydrogen while converting organic substrates into volatile fatty acids and alcohols (Kumar & Das, 2001; Yokoi, Tokushige, Hirose,

Hayashi & Takasaki, 1998; Fang, Zhu & Zhang, 2006). One of the important end product of dark fermentation is volatile fatty acids (acetic acid, butyric acid etc.). The next step, photo-fermentation by which these soluble metabolites can be used with photosynthetic bacteria (purple nonsulphur bacteria), for hydrogen production resulting in increasing hydrogen production yield (Lee, Klaus, Maness & Spear, 2007; Das & Veziroğlu, 2001; Oh, Seol, Kim & Park, 2004; Uyar, Eroglu, Yücel, Gündüz & Türker, 2008; Benemann,1997; Fascetti, D'addrio, Todini & Robertiello, 1998). Combined dark-light fermentation is an alternative of sequential hydrogen production method where dark and light fermentation take place simultaneously in the same fermentation media (Argun, Kargı & Kapdan, 2009). The problems encountered in combined fermentation are the lower hydrogen formation rates, the difference in the rate of acid formation by dark fermentative organisms and acid utilization by photofermentative organisms. Due to this rate difference, accumulation of end products like organic acids could cause product inhibition in dark fermentation and cease the hydrogen production. Similarly, accumulation of organic acids could cause substrate inhibition to photofermentative organisms and lowers the hydrogen generation. Finally, photofermentative organisms are able to use simple carbohydrates such as glucose obtained during biohydrolysis of carbohydrates by dark fermentation. As a results, the organisms could compete for the available glucose for hydrogen production (Argun & Kargi, 2010; Ozmihci & Kargi, 2010; Sagnak & Kargi, 2011).

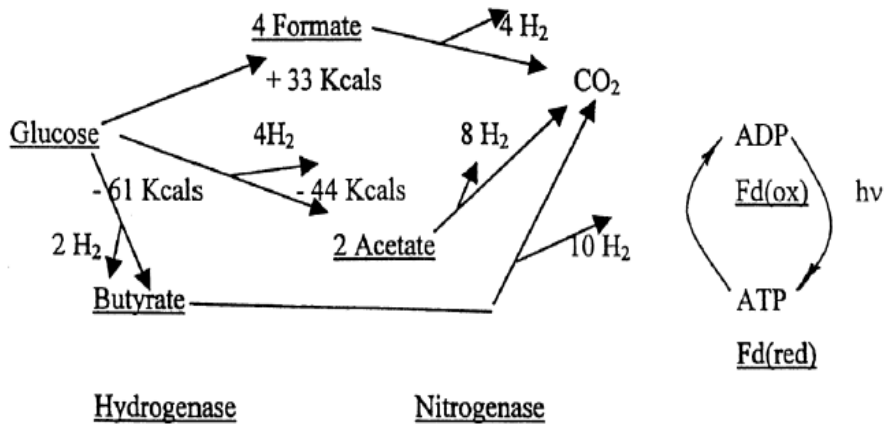


Figure 1.8 Hydrogen production in combined dark and light fermentation from glucose (Das & Veziroğlu, 2001).

1.6 Major Parameters of Hydrogen Production by Dark Fermentation

Environmental conditions are important factor for efficient dark fermentation. The main factors that affect fermentative hydrogen production are as follows;

- Temperature
- pH
- Oxidation Reduction Potential (ORP)
- Hydraulic retention time (HRT)
- Substrate and biomass concentration
- Nutrients
- Microorganism type
- Immobilization
- Types of dark fermentation bioreactors

Temperature is a target operation parameter because, it significantly affects the growth rate and metabolic activity of microorganisms (Lee, Lin & Chang, 2006). Dark-fermentation processes can be operated at mezophilic (25 - 40 °C), thermophilic (40 – 65 °C), extreme thermophilic (65 – 80 °C or > 80 °C) conditions (Levin & Chahine, 2010). The optimal temperature for hydrogen production by mixed culture systems depends on the presence of more complex bacterial populations (Yu, Zhu, Hu & Zhang, 2002; Zhang, Liu & Fang, 2003). It has been demonstrated that the increasing temperature could increase the ability of hydrogen production. But, temperature at much high levels especially beyond mezophilic range could decrease the production yield (Wang & Wan, 2008).

Hydrogen production yield, biogas content, spesific hydrogen production rate and type of organic acids are affected by pH. Because of this reason, pH is crucial for the dark fermentative hydrogen production (Kapdan & Kargı, 2006). Optimal pH range for dark fermentation has been reported as between 5.0-6.0 (Fang & Liu, 2002; Chen, Lin & Chang, 2001).

The oxidaction reduction potential (ORP) is important to provide anaerobic conditions. Since, the hydrogenase enzyme is very sensitive to oxygen inhibition, ORP has to be kept at < -150 mV in dark fermentative hydrogen production processes (Argun, 2010). In order to eliminate oxygen and to get ORP = -150 mV, liquid phase can be sparged with argon or N_2 gas or by adding reducing chemicals such as Na-thioglycolate or L.cysteine to the liquid phase.

Hydraulic retention time (HRT) is a parameter defined as the average length of time that a soluble compound remains in a bioreactor. It is a significant control parameter in a continuous hydrogen production process. Because, HRT control accumulation of organic acids which inhibits the production if it reaches the high levels, hydrogen utilization by hydrogen-consumers, such as methanogens and also homoacetogens, and finally bioconversion rate of organic substance to both hydrogen and organic acids (Chen, Lin & Chang, 2001).

Substrate and biomass concentration or the biomass / substrate ratio is an another parameter affect the hydrogen production rate and yield. While low subsrate concentrations result in low rate, high substrate concentrations causes inhibition of production. In the same way, low biomass concentrations cause long lag times and slow fermentation rates and high biomass concentrations cause consuming the end products so have an negative effects on fermentation yields and rates. Biomass concentration also affected hydogen production yield and total volatile fatty acid formation (Argun et al., 2008).

Several substrates have been used for fermentative hydrogen production. Simple sugars, starch containing wastes, cellulose containing wastes, food industry wastes and wastewater and waste sludge are commonly used in dark fermentation process as a substrate (Kapdan & Kargı, 2006). Some substrates have a complex structure and they are not appropriate for fermentation but after being pretreated by some methods (sterilization, sonification, acidification, freezing, thawing etc.). They can be easily used by microorganism after pretreatment (Wongthanate, Chinnacotpong & Khumpong, 2014). Biomass is one of the most abundant renewable resources for biohydrogen production (Ni, Leung Y.C., Leung K.H. & Sumathy, 2006). It is inexpensive, clean and reliable (Kapdan & Kargı, 2006). About 12 % of today's

world energy supply is received from biomass, while in many developing countries it contributes 40 –50 % energy supply (Demirbas, 2001). The recent trend is utilization of waste biomass as raw material for hydrogen gas production. Lignocellulosic materails are the primary candidate as biomass.

Apart from carbon source microorganisms need also nitrogen, phosphate and other inorganic minerals for optimal cell cultivation. The nutrients are vital for hydrogen production, the growth and cellular activities of dark fermentative bacteria. Nitrogen is a very important components of proteins, nucleic acids and enzymes and it is the most essential nutrients for hydrogen producing bacteria (Wang & Wan, 2008). Ammonia nitrogen (NH_4Cl), urea (CH_4N_2O), ammonium bicarbonate (NH_4HCO_3), yeast extract and polypepton are the current nitrogen sources for hydrogen production. (Bisaillon, Turcot & Hallenbeck, 2006; Salerno, Park, Zuo & Logan, 2006; Argun et al., 2008; Lay, Fan, Hwang, Chang & Hsu, 2005; Liu & Shen, 2004; Morimoto et al., 2004; Yokoi, Ohkawara, Hirose, Hayashi & Takasaki, 1995). Phosphate is also necessary for hydrogen production because its nutritious value as well as buffering capacity. Appropriate concentration of phosphate increase the hydrogen production by dark fermentative bacteria, but phosphate concentrations at much higher levels cause inhibition of hydrogen production (Bisaillon, Turcot & Hallenbeck, 2006 ; Lay et al., 2005; Argun et al., 2008).

Metal ions are considered to be toxic at higher concentration, but a trace level of metal ion is required for fermentative hydrogen production. Mg^{2+}, Na^{+}, Zn^{2+}, Fe^{2+} are considered as the essential ions for cultivation of organisms and for hydrogen production (Li & Fang, 2007).

Microorganism type is another important parameter for biohydrogen production. There are many types of microorganisms that have been used for dark fermentative hydrogen production. Pure cultures may be used to obtain high yield of hydrogen. However, pure culture utilization reveals some drawback primarly sterilization requirement. Therefore, it is not practical and economical hence not applicable in industrial scale. Pure cultures can be easily contaminated by the other bacterial cultures from feed and other sources. Therefore, utilization of pure culture does not represent the real conditions (Hallenbeck et al., 2009).

The major bacterial culture which are used in hydrogen production are *C.butyricum* (Yokoi et al., 2001), *C.thermolactium* (Collet et al., 2004), *C.pasteurianum* (Lin & Lay, 2004; Lıu & Shen, 2004), *C.paraputrificum* M-21 (Evvyernie, 2001) and *C.bifermentants* (Wang et al., 2003) which belong to genus *Clostridium.* They are obligate anaerobes and spore forming organisms. Apart from *Clostridium* sp., can be obtained by heat treatment of anaerobic sludge (Kapdan & Kargı,2006). *Enterobacter aerogenes* and *E.clocae* ITT-BY08 are some of enteric facultative bacteria. *T. Thermosaccharolyticum, Desulfotomaculum geothermicum, Thermococcus kodakaraensis* KOD1*, Klebisalle oxytoca* HP1 are some thermophilic microorganisms used for biohydrogen production (Kapdan & Kargı, 2006). *E. coli* and *Hafnia alvei* can produce biohydrogen from glucose (Podesta' et al.,1997). *Aeromonos* spp., *Pseudomonos* spp. and *Vibrio* spp. are aerobic cultures can produce hydrogen. *Clostridium* spp. *Actinomyces* spp. and *Porphyromonos* spp. exist in anaerobic granular sludge (Kapdan & Kargı,2006). In mezophilic acidogenic cultures *Thermotogales* species and *Bacillus* sp have been used for hydrogen production (Shin, Youn & Kim, 2004). Some anaerobic thermophilic organisms have a capacity of hydrogen production such as *Thermoanaerobacterium* sp. (Shin, Youn & Kim, 2004; Ueno, Haruta, Ishii & Igarashi, 2001). *T. thermosaccharolyticum* and *Desulfotomaculum geothermicum* can produce hydrogen gas in thermophilic acidogenic culture (Shin, Youn & Kim, 2004). Besides the mezophilic and thermophilic organisms, hyperthermophilic species have a potential ability for producing hydrogen. *Thermococcus kodakaraensis* KOD1 is an example of hyperthermophilic bacteria (Kanai et al., 2005). *Klebisalle oxytoca* HP1 and *Clostridium thermolacticum* can produce hydrogen at different temperatures, 58 ºC and 35 ºC, respectively. (Collet et al., 2004; Minnan et al., 2005). In summary, *Clostridium* sp. and *Enterobacter* sp. are the most commonly used microorganisms as inoculum for biohydrogen production by dark fermentation (Wang & Wan, 2009). *Clostridium* sp. are gram-positive, rod-shaped, strict anaerobes and endospores formers. *Enterobacter* sp. are gram-negative, rod-shaped and facultative anaerobes (Li & Fang, 2007).

Immobilization in biotechnology is defined as "the technique used for the physical or chemical fixation of cells, organelles, enzymes, or other proteins (*e.g.*

monoclonal antibodies) onto a solid support, into a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continued use" (Azbar & Kapdan, p.227).

Immobilization provides significant increase in volumetric hydrogen production in immobilized bioreactors. Higher biomass concentration and higher dilution rates without biomass washout are the main advantages of immobilized cell systems (Zhang, Show, Tay, Liang & Lee, 2008). Improved biological stability, product stability and and product yields are the other advantages of this systems. The effectiveness of the immobilization varies with the cell type and support material type (Azbar & Kapdan, 2012). Figure 1.9 shows classification of cell immobilized systems.

Figure 1.10 illustrates basic immobilization techniques. Attachment, entrapment, containment and aggreregation are techniques for immobilization. Attachment is one of the oldest and simplest immobilization technology. Sand and some active porous materials such as coke, wood, etc. are used for providing immobilization (Vega, Clausen & Gaddy, 1998). Macro and micro porous materials could be used for the entrapment of cells. Brick, ceramics, sintered glass, activated carbon, reticulated foam, stainless steel wire, etc. are some examples of macro and micro porous materials (Azbar & Kapdan, 2012).

Cells might be immobilized by containment behind a barrier, either preformed (hollow fiber systems) or formed (microcapsules and two-phase entrapment) in situ. The barrier might be as simple as interface between to immiscible liquids in which case the cell suspension is emulsified with an organic solvent and re-suspended as droplets in an aqueous phase or may include semi permeable membranes used for microfiltration and ultrafiltration (Rupp, 1985; Chang, 1987).

Self-aggragation is also called 'cell to cell atthacment' technique. Self-aggregation is support particle free, cost effective and more applicable to the large scale hydrogen production systems. The effective size or density of the cells are significantly increased by aggregation. By this way, flocculated cells in the form of large aggragates can be considered to be immobilized (Azbar & Kapdan, 2012).

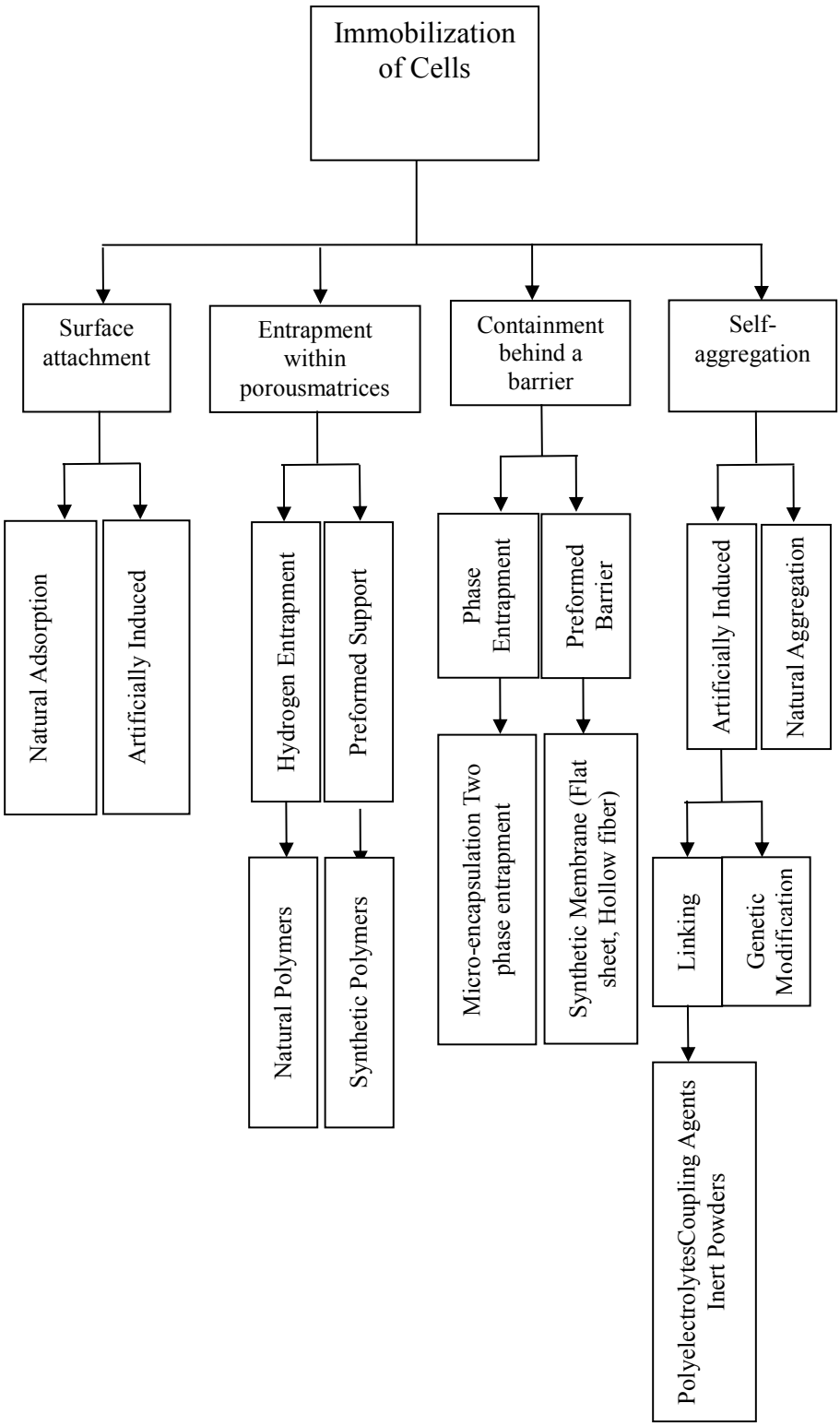


Figure 1.9 Classification of cell immobilized cell systems according to the physical localization and the nature of micro environment (Nedovic & Willaert, 1996)

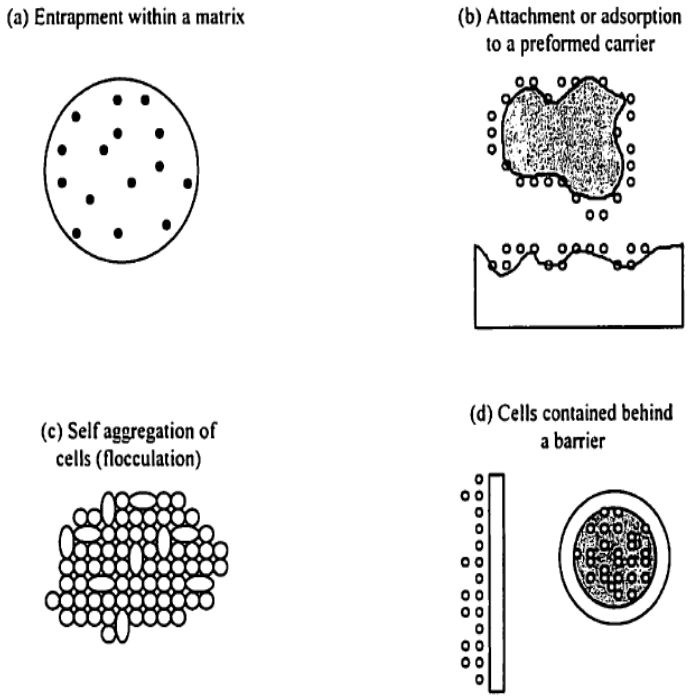


Figure 1.10 Basic techniques of cell immobilization (Pilkington, Margaritis, Mensour & Russel, 1998).

1.7 Literature Review

Hydrogen production studies have been mainly conducted in batch mode to define the production mechanism, selection of bacterial culture and process conditions for a long period of time. Batch mode is preferable by researchers because its requires simple operation and controls. The recent studied have been devoted to continuous operations to determine operation conditions. Suspended or immobilized culture processes have been used so far. Reactors that can be used for hydrogen production are shown in Figure 1.11. The types of reactors that have been used so far are continuous stirred tank reactor (CSTR), up-flow anaerobic sludge blanket bioreactor (UASB), anaerobic sequencing batch reactor, anaerobic biofilm fludized bed reactors, anaerobic granule fludizied bed reactors, carrier-induced granular sludge bed bioreactor, powder activated carbon-assisted agitated granular sludge bed reactor, fixed-bed bioreactor, membrane bioreactor and immobilized-cell continuously stirred anaerobic reactor (Wang & Wan, 2009). Each reactors have different properties. The results revealed that immobilized systems are superior over

suspended culture systems and packed bed reactors are a good alternative for the production of hydrogen because of their simple construction and increased cell retention time (Leite, Fernandes, Pozzi, Barboza & Zairat, 2008; Lin, Wu & Chang, 2006).

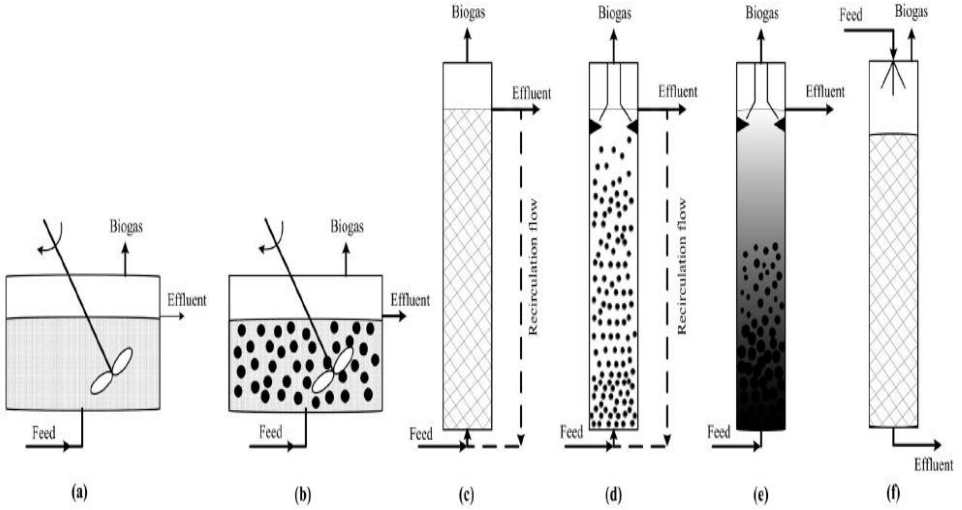


Figure 1.11 Hydrogen-producing reactors schemetic diagram (a) conventional containing suspended sludge CSTR), (b) immobilized-cell CSTR, (c) fixed/packed-bed reactor, (d) fludizied bed reactor, (e) trickling biofilter (Wu, Chang CF & Chang JS, 2007).

Biological hydrogen production from renewable source as known green technology provides sustainable development and waste minimization (Kapdan & Kargı, 2006). In recent years, clean energy sources from waste materials are preferred, due to it contributes clean environment and waste management. Wastes using for biohydrogen production could be biomass, domestic and agricultural wastes. These type of raw materials must be non-toxic, rich carbohydrate content, available, renewable, fermentable and cheap. Barley straw, barley grain, corn stalk, corn grain, sugar beet, ground wheat, sugarcane bagasse have been used for biohydrogen production (Argun et al., 2008; Guo, Fan SQ, Fan YT, Pan & Hou, 2010). One of the most suitable wastes for biohydrogen production are cellulose and strach containing wastes (Sagnak, Kargı & Kapdan, 2011). Some wastes need to be pretreated before using as substrates for biohydrogen production. Processing of ligno-cellulosic compounds is more difficult and comprehensive than the processing of starch (Kapdan & Kargı, 2006). Starch containing agricultural wastes have advantages over raw material because they contain high carbohydrate, nitrogen and

phosphorus and mineral (Fe, Zn, Mg). It was reported by Sagnak, Kargı & Kapdan (2011) waste wheat is one of the most convenient and cheap substrates due to its high starch and gluten content (> 90 %). The annual world wheat-feed production is about 96 million of tones, which is a considerable amount to select this waste as a resource for bio-hydrogen production (Hussy et al., 2003). The characteristics of drum wheat is given in Table 1.5. One of the remarkable point of biohydrogen production from waste wheat is supplementation of media by external nitrogen, phosphorous, Fe (II), Mg (II) and minerals for effective bio-hydrogen production by dark fermentation (Argun, 2010).

Table 1.5 Composition of wheat (Adopted from United States Department of Agriculture, 2009)

Nutrient	Units	Value per 100 grams
Water	g	10.940
Energy	kcal	339
Energy	kJ	1418
Protein	g	13.680
Total lipid (fat)	g	2.470
Ash	g	1.780
Carbohydrate, by difference	g	71.130
Minerals		
Calcium, Ca	mg	34
Iron, Fe	mg	3.520
Magnesium, Mg	mg	144
Phosphorus, P	mg	508
Potassium, K	mg	431
Sodium, Na	mg	2
Zinc, Zn	mg	4.160
Copper, Cu	mg	0.553
Manganese, Mn	mg	3.02
Selenium, Se	µg	89.400
Vitamins		
Vitamin C, total ascorbic acid	mg	0.0
Thiamin	mg	0.419
Riboflavin	mg	0.121
Niacin	mg	6.738
Pantothenic acid	mg	0.935
Vitamin B-6	mg	0.419
Folate, total	mcg	43
Vitamin B-12	mcg	0.00

Various organic wastes such as corn straw, corn stover, cornstalk, grass silage, maize leaves, rice bran, sweet sorghum plant, sugarcane bagasse, *Siplhium trifoliatum* leaves, wheat straw, wheat bran, wheat powder, cow feces and urine, cattle manure, cattle wastewater, dairy manure, pig slurry, swine liquid manure, rice, carrot, cabbage, chicken skin, egg, lean meat, food waste, kithcen waste, molasses, sweet lime peelings extracts, bean curd manufacturing waste, cheese whey, palm oil mill effluent have been used for fermentative bio-hydrogen production as substrate. (Li & Chen, 2007; Datar et al., 2007; Zhang et al., 2007; Karlsson, Vallin & Ejlertsson, 2008; Ivanova, Rakhely & Kovacs, 2009; Noike & Mizuno, 2000; Fan, Zhang Y., Zhang S., Hou & Ren, 2006; Yokoyama et al., 2007; Tang et al., 2008; Xing, Li, Fan & Hou, 2010; Kotsopoulos, 2009; Zhu, Miller, Li & Wu, 2009; Okomoto, Noike, Miyahara & Mizuno, 2000; Li, Zhao, Guo, Qian & Niu, 2008; Argun, Kargı, Kapdan & Oztekin, 2008; Kim, Han & Shin, 2004; Lay, Fang, Hwang, Chang & Hsu, 2005; Shin & Youn, 2005; Kim&Shin, 2008; Wang&Zhao, 2009; Jayalakshmi, Joseph & Sukumaran, 2009; Aceves-Lara, Latrille, Bernet, Buffiere & Steyer, 2008; Chang et al., 2008; Venkata Mohan, Lenin Babu, Venkateswar Reddy, Mohanakrishna, Sarma, 2009; Venetsaneas, Antonopoulou, Stamatelatou, Kornaros & Lyberatos, 2009; O-Thong, Prasertsan, Intrasungka, Dhamwichukorn & Birkeland, 2008). Agricultural wastes are abundant, cheap, renewable and highly biodegradable so biohydrogen production from agricultural waste is promosing approach to hydrogen production (Guo, Trably, Latrille, Carrere & Steyer, 2010).

Wheat is rich in carbohydrate and thus, a good source of glucose for hydrogen production. Several studies have been conducted for hydrogen generation from wheat. Argun & Kargı (2009) investigated batch dark fermentative hydrogen production from waste ground wheat solution. Different sludge pre-treatment methods for selection of active hydrogen producing microbial culture were investigated. The suspended and granular anaerobic sludge obtained from anaerobic treatment plant of the PAKMAYA Bakers Yeast Company (PAK) and wastewater treatment plant of EFES Beer Industry, respectively. The selection of hydrogen producing organisms was performed by repeated heat application for 10 hours (2x5), chloroform and combinations of heat and chloroform pre-treatment. It was reported that heat treatment is the most effective pretreatment method to obtain hydrogen

producing inoculums. The highest hydrogen formation and spesific hydrogen production rate were 652 mL and 25.7 H_2/ g cells h.

The effects of initial substrate (wheat powder-starch) on the biohydrogen rate and the yield were investigated by Argun et al. (2008). For biohydrogen production batchwise fermentative powdered wheat solution was used as substrate and heat-treated anaerobic acidogenic sludge as the inoculum at initial pH of 7.0 and temperature of 37 °C. Initial wheat powder concentration was varied between 5 and 30 g/ L at a constant initial biomass concentration (2.6 g/ L) in order to determine optimal wheat powder concentrations for the highest hydrogen yield. The highest rate and yield of hydrogen production were obtained with an initial substrate content of 20 g/ L. Researchers observed that the low substrate concentration cause substrate limitations and high substrate concentration result in substrate and product inhibitions.

Batch studies with wheat powder indicated that it can be used as raw material for hydrogen production. For continuous hydrogen production Kargı & Pamukoglu (2009) investigated the performance of fed batch operation in hydrogen production from wheat starch. Heat-treated anaerobic sludge was used as inoculum, feed wheat powder solution concentration was varied between 10 - 30 g/ L, wheat powder loading rates were 2 and 4 g wheat powder/ d and feed flow rate was keep constant at 200 mL/ d. Cumulative hydrogen production reached up to 3600 mL at optimal substrate concentration of 20 g/ L. Hydrogen yield was 465 mL H_2 /g starch (3.1 mol H_2/ mol glucose) and hydrogen production rate was 864 mL H_2/ d.

Argun & Kargı (2010) studied on hydrogen production from ground wheat starch by continuous combined fermentation using annular-hybrid bioreactor. The system was operated at six different hydraulic retention times (HRTs). They investigated affects of hydraulic retention time (HRT) on the rate and yield of hydrogen gas formation. The operation was started with 5 g/ L wheat powder with some nutrient supplement. Hydrogen formation decreased with increasing HRT. For effective biohydrogen production by combined fermentation, the system should be operated at HRTs longer than 5 days.

Biohydrogen production from acid treated rice straw hydolysate in both batch and continuous systems was studied by Liu et al. (2013). Concentrated sulphuric acid (55 %) was used for hydrolysis at temperature of 40 ºC. In a batch mode, the effects of initial pH and substrate concentration on hydrogen production at mezophilic temperature (37 ºC) were evaluated. Initial pH of the reactor was varied between pH= 5 to pH= 9 with an initial substrate concentration of 20 g total sugar/ L. The maximum hydrogen production (97.30 ± 0.17 mL/ 100 mL) was obtained at pH 7 with the hydrogen production yield of 0.44 mol H_2/ mol total sugar. Five different initial substrate concentrations (10, 15, 20, 25, 30 g total sugar/ L) were investigated. The optimal biohydrogen yield of 0.41 mol H_2/ mol total sugar was obtained at 20 g total sugar/ L. Then the process was operated at these optimized conditions continuously in CSTR at T= 37 ºC with food industry wastewater at 20 g total sugar/ L. The average hydrogen production yield (0.69 mol H_2/mol T-sugar) obtained in this study was 1.5 times higher than that in the batch system. It was concluded that continuous operation resulted in higer production yield.

Effects of different initial N/ C, P/ C ratios for dark fermentative hydrogen production of sugar solution obtained from acid hydrolysis wheat starch were studied by Oztekin, Kapdan, Kargı & Argun (2008). The powdered wheat was autoclaved at pH=3 and 15 minutes in 90 °C. External addition of nitrogen (N) and phosphorus (P) were necessary to improve hydrogen production rate. However, it was showed that the low nitrogen (N/ C < 0.01) and phosphorus (P/ C < 0.01) contents and excess addition of these nutrients result in low hydrogen production yield. The optimal ratios of the nutrients for maximum hydrogen yield and the maximum specific hydrogen production rate are N/ P/ C=2/ 0.8/ 100 and N/ P/ C=2.5/ 0.8/ 100, respectively.

Some other studies concentrated on utilization of immobilized cell systems for hydrogen production. The significant parameters in continuously operated reactors are hydraulic retention time, initial substrate concentration and immobilization technique or microbial support material type with high biomass holding capacity.

Wu et al. (2002) studied on the effects of immobilized cell systems type on hydrogen production. Cell immobilization was obtained by gel entrapment method

which modified by addition of activated carbon (AC), polyurethane (PU), and acrylic latex plus silicone (ALSC). Hydrogen production in immobilized system was compared with the suspended growth system. Immobilized system had a good performance in comparison to suspended system. Alginate gel supplemented with activated carbon (expressed as CA/AC cells) was superior over other immobilization materials. Polyurethane and acrylic latex silicone addition enhanced mechanical and operational stability of the immobilized cells but has a negative effect on hydrogen production. Hydrogen production rate was 25 times for CA/AC, 10-15 times for PU and ALCS cells higher than that of suspended systems. It was concluded that CA/AC cells may contain higher concentration of active biocatalysts for hydrogen production in comparison to that of PU and ALSC.

Fernandes et al. (2013) investigated the effect of the support material used for biomass immobilization on biohydrogen production in an up-flow anaerobic packed bed reactor. Expanded clay, vegetal coal and low-density polyethylene were used as support material. The synthetic wastewater was composed of sucrose with chemical oxygen demand of approximately 1.000 mg/L. Hydraulic retention time was varied between 0.5 to 2 h. The maximum hydrogen production was observed at HRT 2 h after 10 days of operation and at HRT of 0.5 throughout the 25 day experiment. Decreasing the HRT from 2 to 0.5 h increased the hydrogen yield from 5 mol H_2/mol sucrose to 7 mol H_2/ mol sucrose. It was concluded that there was no substantial difference between the particle types in terms of yield of production. However, when the porosity of the particles mainly low-density polyethylene, the rate of production increased 1.5 times.

Singh et al. (2013) investigated biohydrogen production using immobilized mixed culture in upflow anaerobic sludge blanket (UASB). In this work, palm oil mill effluent (POME) and palm oil mill wastewater treatment plant sludge were used as the substrate and mixed culture, respectively. Polyethylene glycol (PEG) was selected as immobilization surface. POME sludge was entrapped in PEG for continuous hydrogen production. UASB reactor was operated at different hydraulic retention times between 1 h to 12 h with a substrate concentration of 20 g COD/ L to determine their effects on hydrogen production. A suspended growth system was

operated in paralel to UASB to be able to compare the performances of suspended and immobilized systems. The optimal HRTs for immobilized and suspended-cell reactors were 2 and 6 h, respectively. Hydrogen content in biogas of two reactors varied between 12 % - 39.1 % for both systems. In immobilized-cell system, maximum hydrogen production rate was 0.589 L H_2/ L POME/ h at HRT=2 h. The suspended-cell reactor was able to produce hydrogen at maximum rate of 0.348 L H_2/ L POME h at HRT= 6 h. When the immobilized-cell containing reactor was scaled up to 5 L, the hydrogen production rate was 0.500–0.588 L H_2/ L POME h for HRT= 2–10 h, but after the reactor was treated thermally at 60 ºC for 1 h, the rate increased to 0.632 L H_2/ L POME h at HRT 2 h. Butyric acid, acetic acid, propionic acid and ethanol were the main soluble metabolites.

Lay (2000) worked on the conversion of starch into hydrogen by microorganisms in an anerobic digested sludge in a chemostat bioreactor. Heat-treated anaerobic sludge was used as inoculum. Operation temperature was 37°C. pH range varied between 4.7-5.7 while hydraulic retention time (HRT) was 17 hour. If pH was lower than 4.3 and higher than 6.1, alcohol production rate was higher than hydrogen production rate. Maximum hydrogen production rate (1600 L/ m^3/ d) was observed under the organic loading rate of 6 kg starch m^3/ d at pH=5.2 and HRT was 17 hour. Biogas contains approximately 60 % hydrogen, no methanogenesis was exist in the reactor. Maximum hydrogen yield was 2.15 mol H_2/ mol hexose.

Amorim, Barros, Damianovic & Silva (2009) investigated hydrogen production in an anerobic fludizied bed reactor (AFBR) with glucose-based synthetic wastewater as substrate. For biomass immobilization expanded clay was chosen as a support material. Particles of expanded clay were 2.8 - 3.35 mm. Hydraulic retention time for the operation was varied between 8 h to 1 h. The hydrogen production rate increased from 1.41 mol H_2/ mol to 2.49 mol H_2/ mol glucose when HRT decreased 8 h to 2 h. However, for HRT=1 h, hydrogen production yield was 2.41 mol H_2/ mol glucose. Similarly, hydrogen content increased from 8 % to 35 % as HRT was decreases from 8 h to 2 h. It was concluded that expanded clay is an appropriate support material for hydrogen production in AFBRs.

Chang & Lin (2004) aimed to determine the hydrogen production in an up-flow anaerobic sludge blanket (UASB) reactor. Sewage sludge was used an inoculum source for hydrogen producing microorganisms for converting sucrose (20 g COD/L) into hydrogen. Hydraulic retention time (HRTs) was varied between 24 - 4 h. The hydrogen productivity was almost stable at the HRT between 8 h and 20 h. However, productivity substantially decreased for HRT= 4 h and HRT 24 h. Butyrate and acetate were the main volatile fatty acids. Each gram of biomass produced 53.5 mmol H_2/ day with a 42 % hydrogen gas content at a HRT of 8 h. At shorter HRTs (< 8 h), microorganisms tended to washout from reactor. This study showed that UASB reactor can be used for hydrogen production at optimal HRT=8 h.

Leite et al. (2008) operated horizontal-flow anerobic immobilized packed-bed bioreactor with expanded clay beds (4.8-6.3 mm) for the hydrogen and organic acids production from glucose-based synthetic wastewater with HRT = 0.5 h to 2 h. A microbial film was developed without previous inoculation. Alkalinity was one of the most effective parameter and the system produced optimal output when operating without buffer agent. The average hydrogen production was 2.48, 2.15 and 1.81 mol H_2/ mol of glucose for $NaHCO_3$ influent concentrations of 0, 1000 and 2000 mg/ L, respectively.

Lin et al. (2006) reported dark fermentative hydrogen production with a draft tube fludizied bed reactor (DTFBR) containing silicone-gel as an immobilized cell particles from anaerobic sludge. The reactor was operated at different sucrose concentration (5 - 40 g COD/ L) as a substrate, HRT was varied between HRT=2.2 h and 8.9 h, temperature range was 50 to 80 °C. The maximum H_2 production rate was obtained as 2.27 ± 0.131 L/ h/ L at HRT= 2.2 h and 40 g COD/ L. But the highest hydrogen yield was obtained as 4.98 ± 0.18 mol H_2/ mol sucrose at HRT=8.9 h and at 40 g COD/ L was. The hydrogen content in the biogas was above 40 % during operation.

Chang et al. (2002) investigated the effects of loofah sponge (LS), expanded clay (EC) and activated carbon (AC) as immobilization materials and hydraulic retention time (0.5 - 5 h) on hydrogen production from 20 g COD/ L sucrose in continuously operating fixed-bed bioreactors. The reactor was operated at mesophilic conditions

(35 ^{0}C). It was stated that the LS was inadequate for ensuring immobilization. Maximum hydrogen production rate was 0.415 L/ h/ L and obtained at HRT=2 h for reactor contain EC. On the other hand hydrogen production rates with AC was 1.32 L/ h/ L at HRT=1 h.

Wu, Chang C-F & Chang J-S (2007) worked on hydrogen production from dark fermentation using polyethylene-octane elastomer immobilized anaerobic sludge as the biocatalyst in a packed bed reactor. The maximum hydrogen production rates were obtained 10.3 mmol/ h L, 16.1 mmol/ h L, 7.2 mmol/ h L at HRT=4 h for sucrose, glucose and fructose with initial substrate concentration of 20 g COD/ L, respectively.

These studies indicated that hydrogen production yields and rates varies depending on the operation mode batch or continuous, substrate type as complex carbohdrates or simple sugars, immobilized or suspended bioreactor configurations. Simple sugars are advantageous but not cost effective. Complex carbohydrates require hydrolysis step either chemical or enzmatic. Bioreactor configuration is certainly suggested as immobilized system. However, the type of immobilization technique, or support material are the major concern to obtain high hydrogen yield or rates. Depending on this fact, optimal operating conditions as HRT or substrate concentration, process stability etc. vary. Therefore, each process needs self optimization studies based on support particle type and substrate type.

1.8 Objectives and the Scope

The main objective of this study is to investigate biohydrogen gas production in a continuously operated immobilized bioprocess from acid hydrolyzed wheat starch by dark fermentation.

In order to achieve this aim, the scope of the study can be summarized as follows;

- To investigate the effects of hydraulic retention time on biohydrogen gas production rate and yield in continuous dark fermentation

- To investigate the effects of initial substrate concentration on biohydrogen gas production in continuous dark fermentation process containing novel support material (aquarium biological sponge) under mesophilic conditions
- To determine potential of the biohydrogen gas production in continuously operated up-flow packed bed reactor
- To determine the optimal operation conditions for enhanced biohydrogen gas formation rate and yield

CHAPTER TWO
MATERIALS AND METHOD

2.1 Dark Fermentation

2.1.1 Organisms

The mixed microbial culture was obtained from the acidogenic phase of anaerobic wastewater treatment plant of PAK MAYA Bakers Yeast Company in Izmir, Turkey. The concentrated anaerobic sludge was diluted by half with distilled water. The initial pH range of the anaerobic sludge (ANS) measured approximately 7.5. pH was adjusted with diluted sulphuric acid to pH=5.9. Sludge was boiled for 5 h at 100°C in order to select hydrogen producing, spore forming acidogenic bacteria and to eliminate hydrogen consuming methanogens. Heat- pretreated anaerobic sludge was cooled and used as inoculums.

2.1.2 Substrate Preperation

Acid hydrolysis wheat powder (AHWP) was used as a carbon source. Waste wheat was obtained from SOKE wheat flour in Izmir, Turkey. Waste wheat was ground, sieved down to 70 μm size (less than 200 mesh) and then hydrolysed under acidic condition by sulphuric acid with pH=2 and at high temperature (90 ^{0}C for 15 min) in an autoclave. The conversion yield of starch to soluble total sugar was 90% by acid hydrolysis at high temperature. The solid phase of hydrolyzed wheat powder solution was separated by centrifugation at 8000 rpm for 20 minutes. Supernatant sugar solution was neutralized to pH=7 by addition of 10 % NaOH. The nitrogen content of wheat powder solution was not sufficient for fermentation. The wheat powder contained approximately 97 % (w/ w) starch, 3.4 mg/ g total nitrogen and 1.72 mg/ g phosphate-P. For this reason, external KH_2PO_4 and CON_2H_4 addition were required as phosphorus and nitrogen source to overcome nutrient limitations, respectively to the optimal C/ N/ P of 100/ 1/ 0.1 (Argun, 2010). Oxidation reduction potential was adjusted to ORP < -150 mV by adding Na-thioglycolate (200 mg/ L) to the feed in the beginning of the continuous operation for maximum hydrogen production rate. The initial waste powder was 20 g/ L.

2.1.3 Support Material Preperation

Aquarium biological sponge (ABS) was used as an immobilization surface for hydrogen production from hyrolyzed waste powder in continously operated up-flow packed bed bioreactor (UPBR) (Gokfiliz, 2014) . The support material particles size was adjusted to L=1 cm, D=1 cm, W=1 cm. Total number of particle in the reactor was N_p=2650. The Picture of the support particle is given in Figure 2.1.

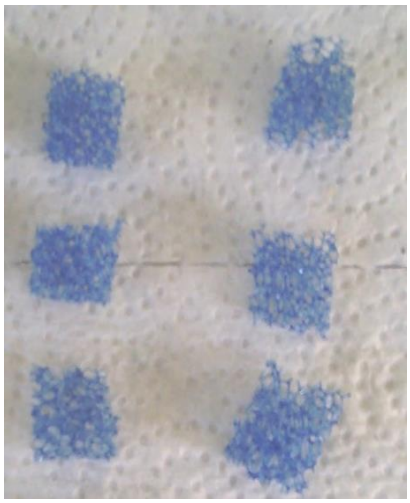


Figure 2.1 Aqurium biological sponge as used for support material with dimension (1^{cm}x1^{cm}x1^{cm})

2.1.4 Bioreactor Configuration

Biohydrogen production from hydrolysed waste wheat powder by dark fermantation was conducted in up-flow packed bed bioreactor. The reactor was made up stainless steel with dimentions of 57 cm in lenght and 8 cm in diameter. Total liquid volume in the reactor was 1400 mL. There was 4 sampling port along the reactor. Substrate was fed from the bottom and the effluent was discharged from 4^{th} port. The hydrogen gas was collected from the top of the reactor and was transferred to gas collection unit via gas tight teflon tubing. Temperature was adjusted to T= 37 ^{o}C by water-bath and heating jacket around the reactor. Figure 2.2 shows experimental set up.

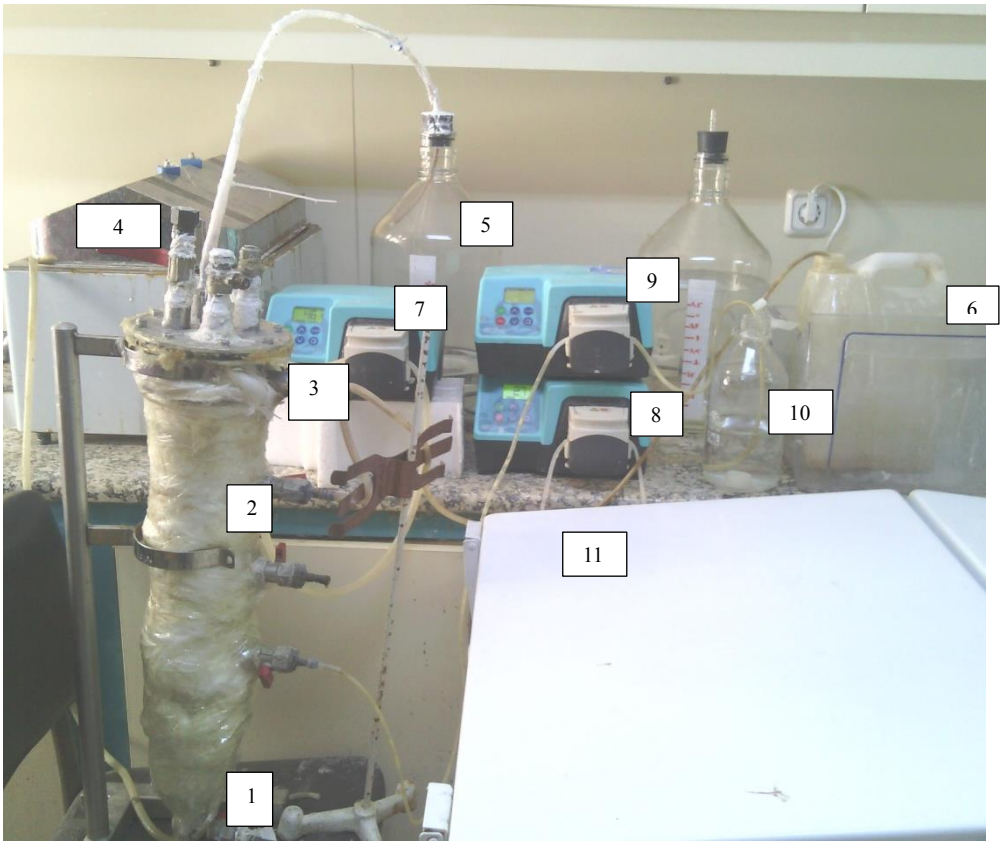


Figure 2.2 Continously operated up-flow packed bed reactor for hydrogen production. (1) influent port, (2) heating jacket, (3) effluent port, (4) water-bath, (5) gas-hold bottle, (6) effluent drum, (7) peristaltic pump for pumping warm water from water-bath to around reactor, (8) peristaltic pump for pumping from influent drum to influent port, (9) peristaltic pump for manuel pH adjustment, (10) 10 M NaOH, (11) deep refrigerator at 4 °C for influent drum in order to avoid any decomposition.

2.1.5 Immobilization of Anaerobic Sludge

In the beginning of the experiments, the reactor was filled up 2650 ABS as support material, 800 mL growth media containing 20 g/ L AHWP and 600 mL heat pre-treated ANS. pH must be adjusted 6.5-7.0. The reactor was operated batch wise liquid recycle for one week to provide initial immobilization of anaerobic sludge. Then, the reactor was operated continuously with inital substrate concentration for at least 10 days to enhance the growth of immobilized microorganisms.

The reactor was inoculated with heat treated anaerobic sludge and then operated batchwise with effluent recycle at 20 g/ L glucose concentration for at least 2 weeks to provide immobilization of the biomass on the support particles. The growth media was refreshed every three days during 2 weeks of batch immobilization period. Then,

the reactor was operated continuosly with hydrolized wheat powder with total sugar concentration of TS_0= 15 g/ L and at HRT= 6 h for 10 days to enhance the immobilization. Hydrogen volume, hydrogen percentage and effleunt sugar concentration and biomass concentration was monitored during continuous operation for start-up purpose. pH and ORP values was controlled at pH= 5.0-6.0 by adding dilute 10 M NaOH and ORP < -150 mV by adding 200 mg/ L Na-thioglycolate to the feed. The experiements were started when hydrogen percentages was more than 20 % and almost no biomass in the effluent.

2.2 Analytical Methods

2.2.1 Sample Preperation

Samples were removed from effluent centrifuged everyday at 8000 rpm for 10 minutes to remove solids from the liquid phase and the clear supernatants were used for total sugar (TS) and total volatile fatty acids (TVFA) analysis.

2.2.2 Total Gas Volume Measurement

Total gas volume was measured everyday. The water displacement method was used for determining the amount of total gas volume produced by using 2 % sulphuric acid and 10 % NaCl containing solution.

2.2.3 Hydrogen Gas Measurement

Hydrogen gas was sampled from the head space of the reactor by using gas-tight glass syringes and hydrogen concentrations of the total gas 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 and the head pressure was 22 psi. Temperatures of the oven, injection, detector, and filament were 35 ºC, 120 ºC, 120 ºC, 140 ºC, respectively.

2.2.4 Measurements of pH and ORP

pH and ORP of the fermentation medium were measured everyday. They were monitored by using a pH meter and ORP meter with relevant probes (WTW Sci., Germany). pH of the reactor decreased from an initial value of 7.0 to nearly 4.5 due

to organic acid production which was adjusted to 5.5-6.0 by manuel pH control with addition of 10 M NaOH everyday. ORP values varied between -100 and -300 mV, in general.

2.2.5 Total Sugar Analysis

Initial and residual sugar concentrations was determined by acid-phenol spectrometric method (Dubois et al.,1956). The samples were analysed in triplicates and results were reproducible within 3 % deviation.

2.2.6 Total Volatile Fatty Acids Analysis

Total volatile fatty acids analysis were mesured by using analytical kits (Spectroquant, 1. 01763. 0001, Merck, Germany) and a PC spectrometer (WTW Photolab S12).

2.2.7 Calculations

The moles of cumulative hydrogen was calculated by using ideal gas law equation:

$$PV = nRT \qquad (2.\ 1)$$

where:

P ;the absolute pressure of the gas (1 atm),

V ; CHV (L)

n ; the moles of CHV (mol)

R ; the ideal, or universal, gas constant, 0.082 (L atm / mol K),

T ; the absolute temperature of the gas (Kelvins ; 273 K = 0.00 ºC).

Hydrogen gas volumes were correlated to room temperature (25 ºC).

Steady-state volumetric (R_v, mL H_2 /L day) rates of hydrogen gas formation was calculated by using the equation 2:

$$R_v = V_{H2}/V \qquad (2.\ 2)$$

where:

V_{H2} : Volume of hydrogen gas formation per day (mL H_2/ day)

V: Volume of fermentation media in the reactor, L

The substrate loading rate was calculated as

$$L_s = Q\, S_0 / V = S_0 / \mathrm{HRT} = D\, S_0 \qquad (2.3)$$

2.3 The Effect Of Environmental Factors

2.3.1 Dark Fermentation Temperature

Anerobic sludge and wheat powder solution was used as inoculum and carbon source, respectively. Hydrogen production by dark fermentation experiments were done in up-flow packed bed reactor. Fermentation was done at mezophilic temperature (35 – 37 °C).

2.3.2 Dark Fermentation pH

pH values was controlled manually with 10 M NaOH and adjusted at pH= 5.0-6.0.

2.4 Experimental Conditions

The effects HRT and initial substrate concentration on hydrogen production rate were investigated. APBR was operated continously at HRT= 1 h, 2 h, 3 h, 4.5 h, 6 h, 8 h. Initial substrate concentrations was kept constant at 15 g/ L. The maximum production was achieved at HRT= 3 hours. Initial substrate concentrations were 10 g/ L, 15 g/ L, 19 g/ L, 25 g/ L, 35 g/ L in the second set of experiment at constant HRT= 3 h. Wheat powder solution was preserved at 4 °C againts to decomposition. Total gas volume, hydrogen gas concentration in total gas, total sugar concentration in the effluent, pH, ORP, temperature were monitored everyday and each were controlled at pH= 5.0-6.0 by adding 10 M NaOH in to the feed, ORP < -150 mV by adding 200 mg/ L Na-thioglycolate to the feed, T= 37 ^{o}C, respectively. Each experiments were conducted until steady state conditions with respect to hydrogen volume were reached.

CHAPTER THREE
RESULTS AND DISCUSSIONS

Hydrogen production studies in immobilized system consist of two parts. In the first part of the study, the system was operated at six different hydraulic retention times in order to select the optimum hydraulic retention time for maximum hydrogen productivity in an up-flow packed bed reactor. In the second part of the study, the effect of initial substrate concentration on hydrogen production at selected HRT was investigated.

3.1 Hydrogen Gas Production in Continuously Operated Up-Flow Packed Bed Reactor

Continuous dark fermentation studies in an up-flow packed bed reactor (UPBR) were carried out at six different HRTs between 1 hour and 8 hours. Aquarium biological sponge was used as support material for immobilization of microorganisms in the reactor. Total particle number in the UPBR was 2650. Initial substrate concentration was kept constant at TS_o=15 g/ L. The system was operated until the steady-state was reached for every study HRTs. Daily total gas volume, total hydrogen gas volume, hydrogen production yield and rate, total volatile fatty acids concentration, effluent total sugar concentration were monitored for every experimental conditions.

3.1.1 Effect of Hydraulic Retention Times (HRTs) on Hydrogen Production

3.1.1.1 Hydrogen Production at HRT=8 h

Up-flow packed bed reactor was first operated at HRT=8 h. Variation of hydrogen gas volume with time was given in Figure 3.1. The daily hydrogen production volume was 143 mL/ day in first day of this set. Steady-state condition was obtained in terms of hydrogen gas production after 4^{th} day. Hydrogen percentage was 33 % ± 2 % in the total gas.

Hydrogen production rate represent the volume of hydrogen produced per day per liquid volume of the reactor. In immobilized systems, the liquid volume is less then reactor volume due to volume occupied by the particles. This parameter is very

important to be able to compare the hydrogen production performances of the process depending on the particle type, operation conditions, reactor type, reactor volume, microbial culture and also environmental conditions. For example, the volume of the production could be very high if the high reactor volumes are used. In other words, any study could report very high hydrogen production volume and claim that the process operates with high performance. However, the process may need very large reactor volume which makes it is infeasible during the full scale production. The rate is also related with substrate loading rate which is the function of substrate concentration and feeding rate (HRT). In most of the cases, higher the substrate loading, better the production due to high concentration of available substrate. The more comparable parameter is specific hydrogen production rate (L H_2/ L/ day) which defines the hydrogen production capability of the microorganism. Generally mixed bacterial cultures are used in hydrogen production by dark fermentation. They are mainly spore forming *Clostridium* sp. selected from mixed anaerobic culture by exposing extreme environmental conditions such as heat or acid treatment. If these selection is well performed than the production rate will be higher. Moreover, the concentration of biomass is also important factor. High biomass concentration will result in high hydrogen production. This is the main reason why immobilized systems are used in hydrogen production. However, it is not practical to determine the total biomass concentration in an immobilized system. Some of the culture is immobilized in micropores and it could be very difficult to extract them from these micropores. By considering these facts, hydrogen production rates rather than specific hydrogen production rates were calculated at each operation conditions. For the first operation condition at HRT=8 h, the rate gradually increased and reached to maximum value of R= 0.71 L H_2/ L/ day (Figure 3.2).

Yield of production is related with how efficiently substrate is converted to hydrogen gas. In other words, if substrate is used for growth, hydrogen production or for other purposes. The main aim is to divert the microbial metabolism to hydrogen production rather than growth or other metabolite production. Because substrate is the main cost of production and should be completely used for product formation. Therefore, the yield of production is calculated in the concept of this study. Figure 3.3 depicts the variation of yield of formation in time at HRT=8 h. It was around

Y=20 mL/ g TS in the beginning of operation and then reached to 248 mL/ g TS at the steady state.

Initial sugar concentration was adjusted to 15 g/ L ± 1 g/ L. During the pre-steady state there was a substantial fluctuation in sugar consumption. Although, the system was operated batch wise to provide immobilization of microorganisms for a certain period of time, it seems that there was still growth of microorganisms and increase in the biomass. Therefore, high rate of substrate utilization with the effluent sugar concentration of around 4 g/ L was observed in the initial state of operation. Sugar consumption by microorganisms reached to steady state after 4^{th} day and effluent concentration of sugar increased to 10 g/ L (Figure 3.4). No further variation in the sugar concentration was observed and resulting substrate removal efficiency was approximately 29 % at HRT=8 h. The consumed sugar was partially used for growth and the rest of it was converted to volatile fatty acids. TVFA concentration was around 1.5 g/ L in the beginning of operation. Although, it slightly increased to 2 g/ L at the end of first day of operation, the steady state concentration of TVFA was around 1.5 g/ L (Figure 3.5). TVFA is the function of available sugar concentration, capability of microbial culture to convert sugar to TVFA, hydraulic retention time, the generated TVFA concentration in the process and environmental conditions as pH. TVFA production at this HRT did not vary substantially although sugar consumption fluctuated. This result indicates that conversion of sugar to TVFA was almost constant, the high rate of sugar consumption at the pre-steady state was due to utilization of sugar for growth.

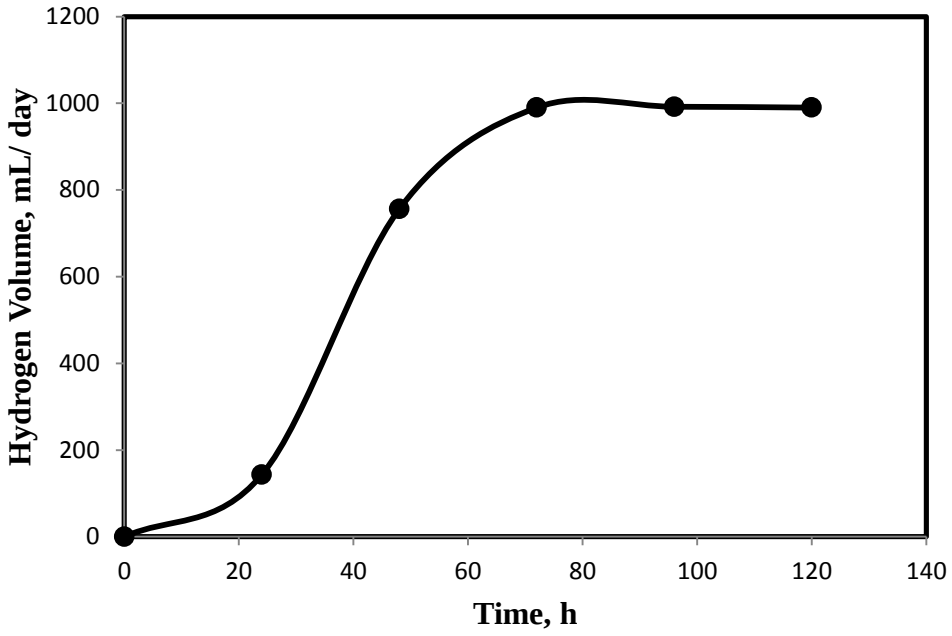


Figure 3.1 Variation of hydrogen gas volume with time at HRT=8 h

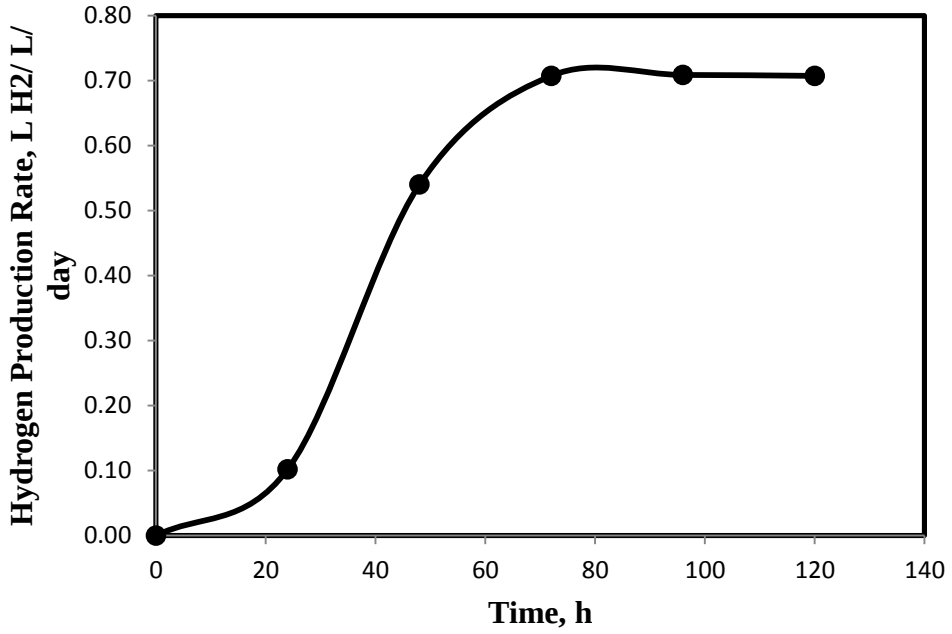


Figure 3.2 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at HRT =8 h

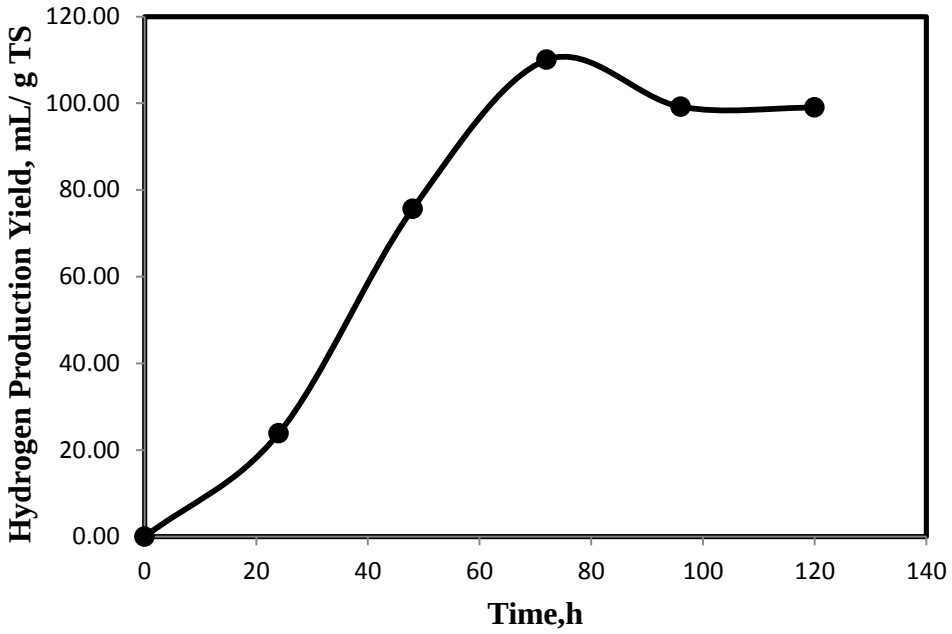


Figure 3.3 Variation of hydrogen production yield (mL/ g TS) with time at HRT =8 h

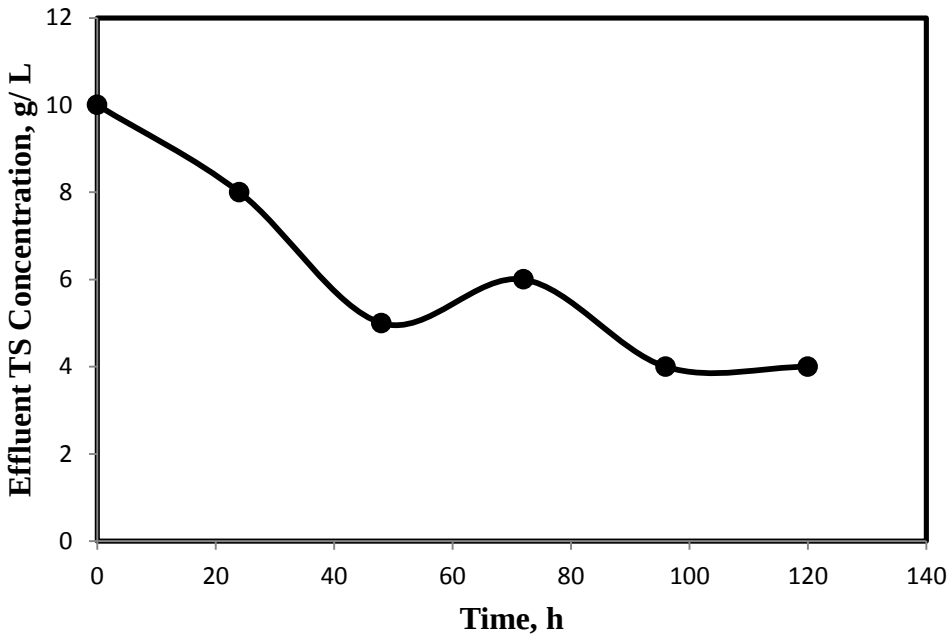


Figure 3.4 Variation of effluent total sugar concentration (g/ L) with time at HRT =8 h

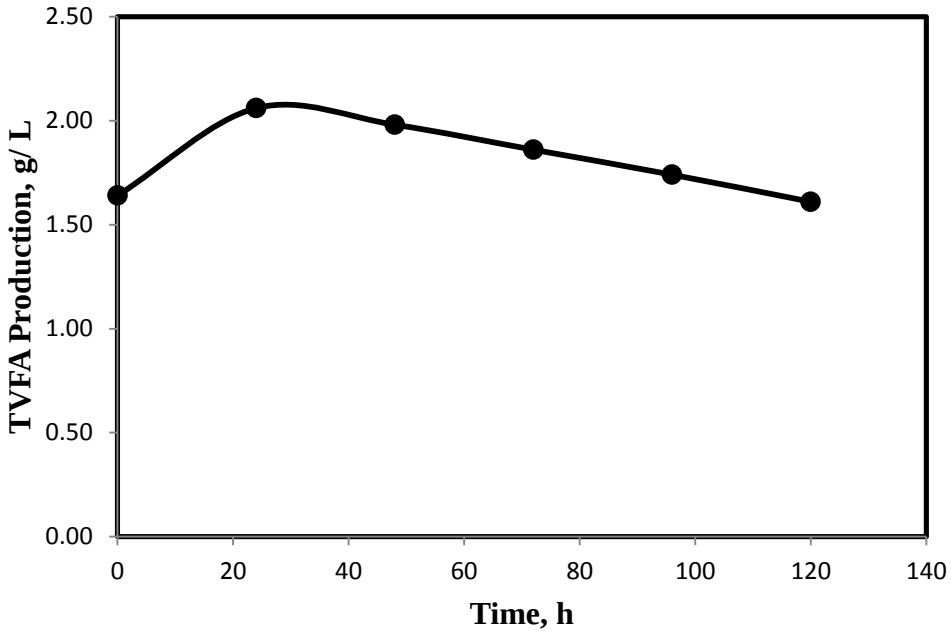


Figure 3.5 Variation of total volatile acids production (g/ L) with time at HRT =8 h

3.1.1.2 Hydrogen Production at HRT =6 h

Hydrogen gas generation by dark fermentation generally desired to occur at short HRTs between 2 - 4 h in immobilized systems. The support particle type is a significant factor to achieve the production in short HRTs. The support particles which retain high biomass, provide high void volume which easy release of gas form liquid phase to head space, and long lasting ones are preferred. Therefore, the optimal HRT for the maximum production could vary depending on the support particle. The aquarium biological sponge has got high porosity which may help both high biomass holding and gas transfers form liquid to head space.

The other factor that affects hydrogen generation is the amount of TVFA. Exposure to high TVFA concentration for a long period of time, limits the microbial activity for further production of TVFA which is directly linked to the hydrogen generation. Longer HRTs provide the conditions as long exposure to produced TVFA. Therefore, generation of hydrogen could be limited by product as TVFA. That's why, HRT was gradually decreased in this study.

Variation of hydrogen gas volume with time at HRT=6 h time is depicted in Figure 3.6. The daily hydrogen volume steadily increased from 500 mL/ day to around 900 mL/ day during 3 days of continuous operation and remained almost constant at this value for the rest of operation. The last three days daily hydrogen gas volumes were almost equal to each other around 924 mL/ day. The percentage of hydrogen gas in the total gas was 66 % in this set of the operation. A kind of hydrogen enrichment in the gas phase was achieved as HRT was decreased from 8 h to 6 h. This enrichment could be result of increase in the linear flow rate in the reactor with the decrease in HRT which is achieved by increasing in feeding rate. As flow increased the hydrogen gas bubbles in the liquid phase or in the inner side of the support particles are forced upwards to the head space. The other explanation is the metabolic shift in the organisms. The metabolism of the organisms could be shifted to TVFA production rather than CO_2 generation which resulted in less CO_2 generation or lower percentage of CO_2 in the total gas. As a result, although the volume of hydrogen was almost equal to each other at HRT = 3 h and HRT = 8 h, percentage of the hydrogen gas in the total gas increased.

Hydrogen production rate did not substantially changed as HRT was decreased from 8 h to HRT=6 h. Maximum hydrogen production rate was R=0.67 L H_2/ L/ day as shown in Figure 3.7. However the yield of formation decreased to Y=94.25 mL/ g TS (Figure 3.8) at HRT=6 h. This could be because of the metabolic shift of organisms from hydrogen generation organic acid production to non-hydrogen generating end products like propionic acid or ethanol. That evaluation can be supported by considering substrate consumption and TVFA concentration. Initial substrate concentration was around 15 g/ L and effluent substrate concentration at steady state was roughly 5 g/ L resulting in 67 % substrate utilization (Figure 3.9). Substrate consumption was considerably higher at this HRT compared to HRT=8 h (29 %). The expectation was conversion of utilized sugar to hydrogen generating volatile fatty acids. TVFA production at steady state was about 1.115 g/ L (Figure 3.10). That concentration is less than what was obtained at HRT=8 h. Since lower TVFA was produced, it is acceptable to get lower yield of formation. Substrate was utilized for other purposes rather than hydrogen generating volatile fatty acid production.

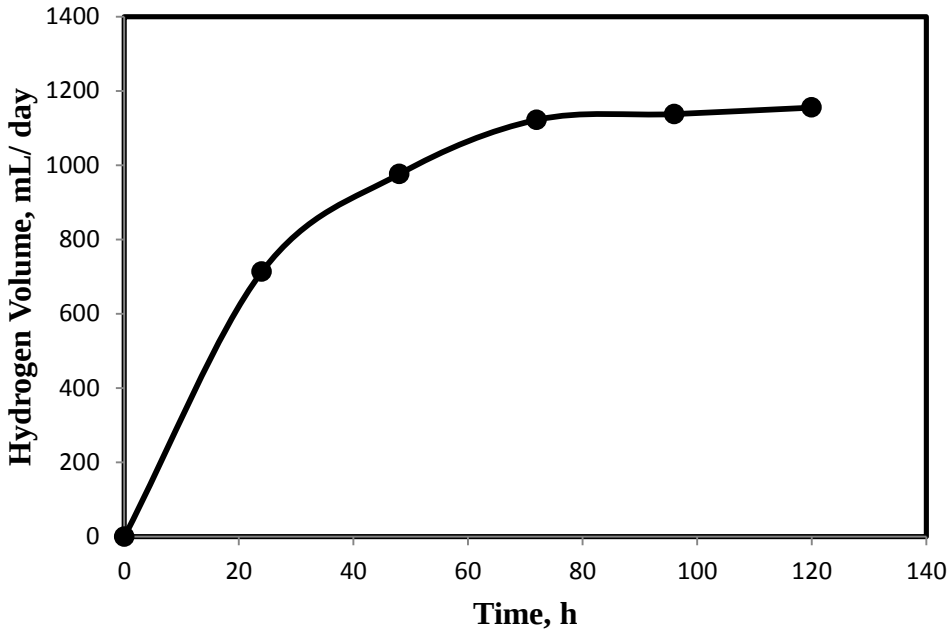


Figure 3.6 Variation of hydrogen gas volume with time at HRT=6 h

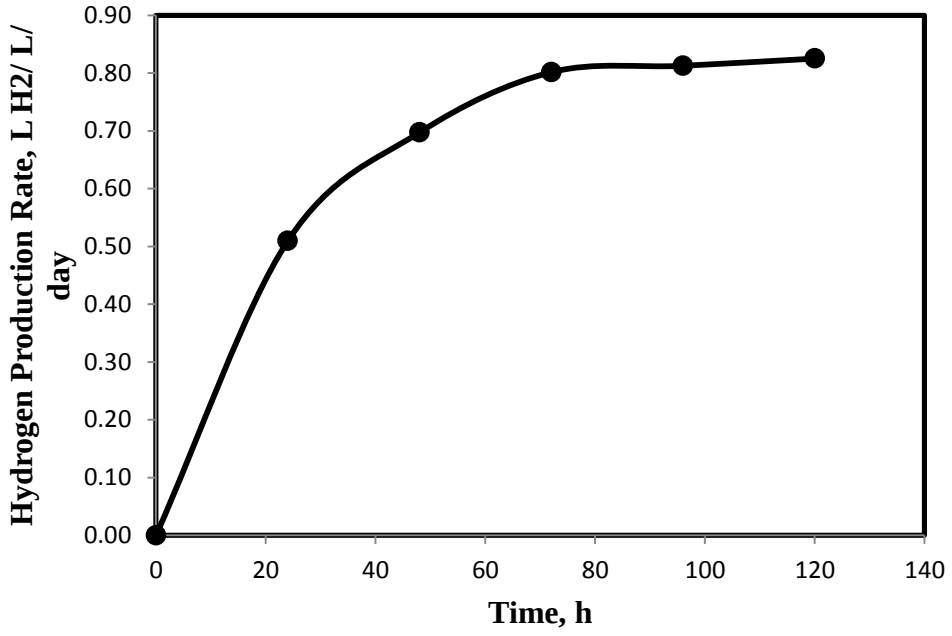


Figure 3.7 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at HRT =6 h

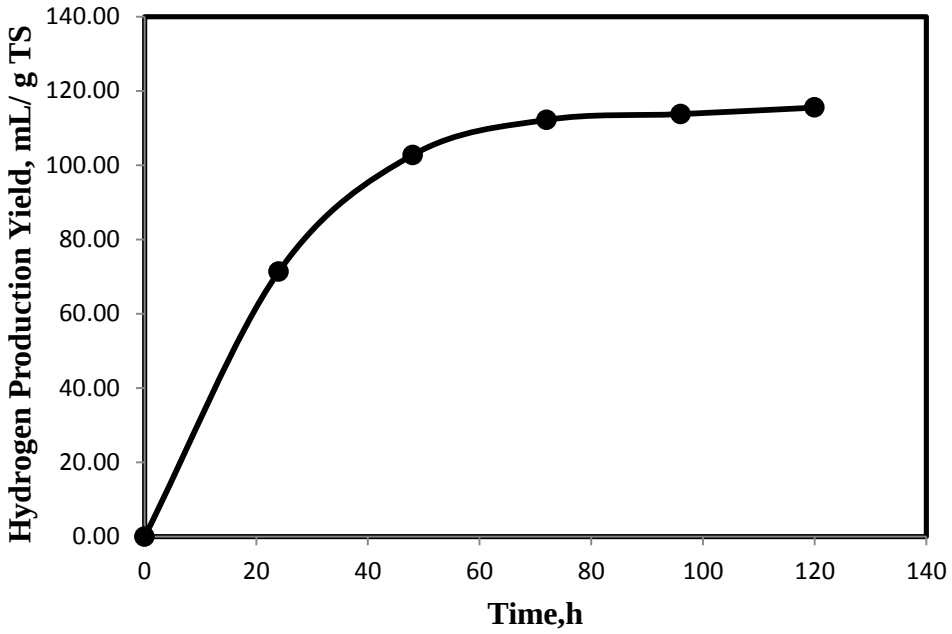


Figure 3.8 Variation of hydrogen production yield (mL/ g TS) with time at HRT =6 h

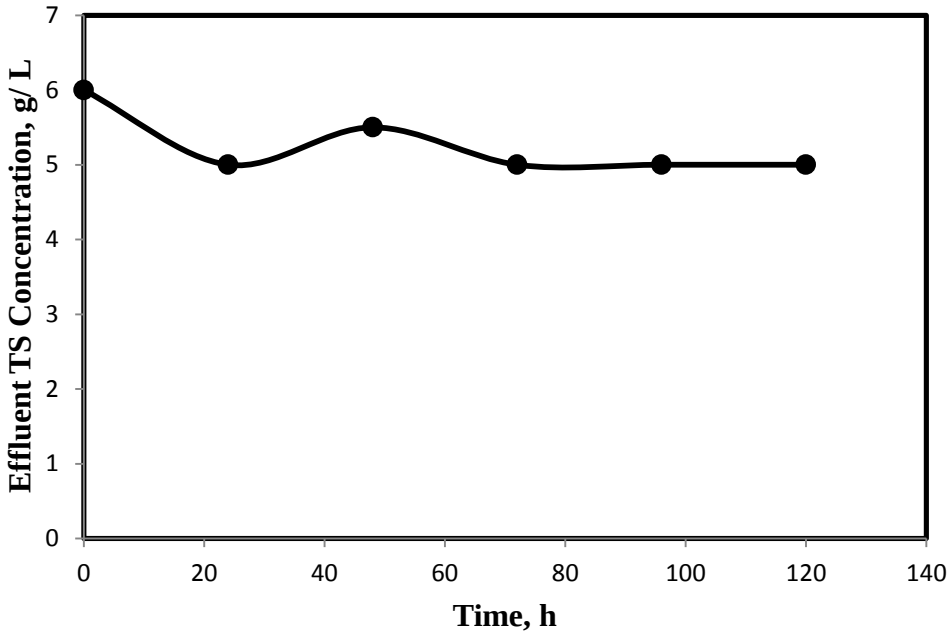


Figure 3.9 Variation of effluent total sugar concentration (g/ L) with time at HRT =6 h

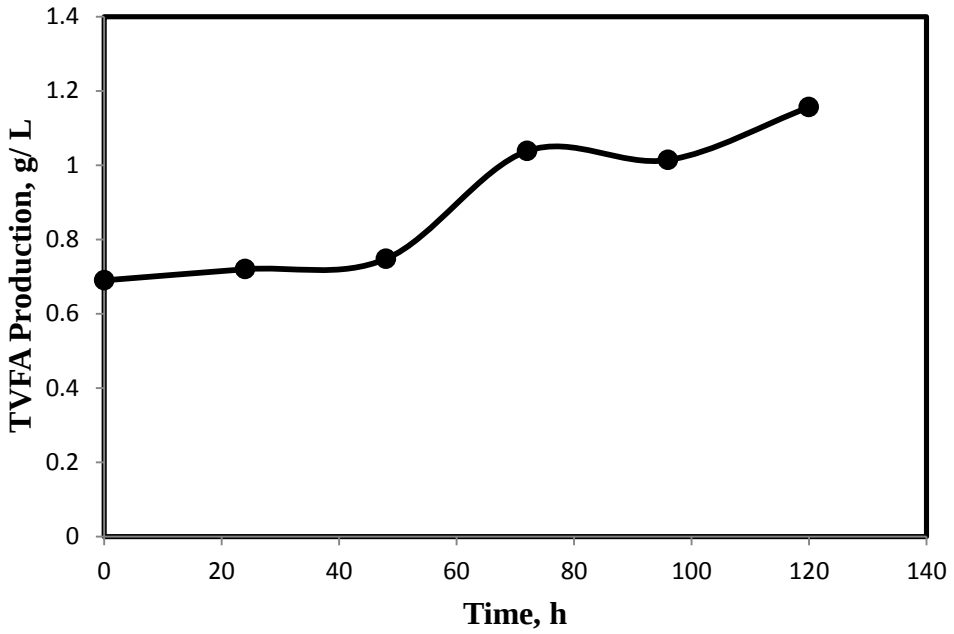


Figure 3.10 Variation of total volatile acids production (g/ L) with time at HRT =6 h

3.1.1.3 Hydrogen Production at HRT=4.5 h

In order to evaluate the effect of lower HRT, the system was operated at HRT = 4.5 h. Variation of hydrogen volume with time is given in Figure 3.11. There was a substantial fluctuation in the hydrogen volume during pre-steady state. About 500 mL/ day hydrogen production was observed up to 45 hours of operation. Then a sharp increase in the volume was achieved and steady state hydrogen volume reached to 1700 mL/ day at the end of 96 h of continuous operation. Hydrogen percentages were measured nearly 61 %. Since volume of production substantially increased at this HRT, higher volumetric hydrogen production rate was obtained as 1.63 L H_2/ L/ day (Figure 3.12) at steady-state condition. That production rate was almost 2.3 times and 2.7 times of the volume obtained at HRT=8 h and HRT=6 h, respectively. Increasing rate of feeding resulted in increasing in substrate loading rate and linear velocity which helped diversion of metabolism to hydrogen production and also separation of hydrogen bubbles produced from liquid phase to the head space of the reactor. The latter one is quite important in hydrogen production. If liquid phase hydrogen concentration increases certain concentrations, the microorganisms divert their metabolism to non hydrogen production conditions due to the condition in which excess amount of product (hydrogen) formed. As it was

explained before, the other product in this process is TVFA. Product inhibition could be observed at certain concentrations (2.5 g/ L) of TVFA. Since feeding rate is high, the discharge rate of these products from the system is high. As a result, the exposure time to produced TVFA is decreased as HRT is shortened. Then, product inhibition is overcame. Similar to production rate, the yield of formation substantially increased as HRT was reduced. Maximum production yields was 456.0 mL/ g TS as shown in Figure 3.13. This results is 1.8 times and 4.9 times higher than ones obtained at HRT=8 and HRT=6 h, respectively.

It is evident that microorganisms need certain contact time with the substrate to able to consume them almost completely. Reducing HRT limits the time needed to utilize all the substrate. Therefore, the expectation was lower substrate utilization at that HRT compared to HRT=8 h and HRT=6 h. The resulting effluent total sugar concentration at steady-state was approximately 10 g/ L (Figure 3.14) and total sugar utilization was 33 %. Although, substrate removal efficiency is low, the amount of TVFA produced was considerably high compared to previously examined HRTs. The steady state effluent TVFA concentration was nearly 5.5 g/ L (Figure 3.15). So, better volatile fatty acid production, more hydrogen generating volatile fatty acid production as acetic acid, butyric acid and finally better hydrogen separation from liquid phase to head space were achieved by decreasing hydraulic retention time to HRT = 4.5 h.

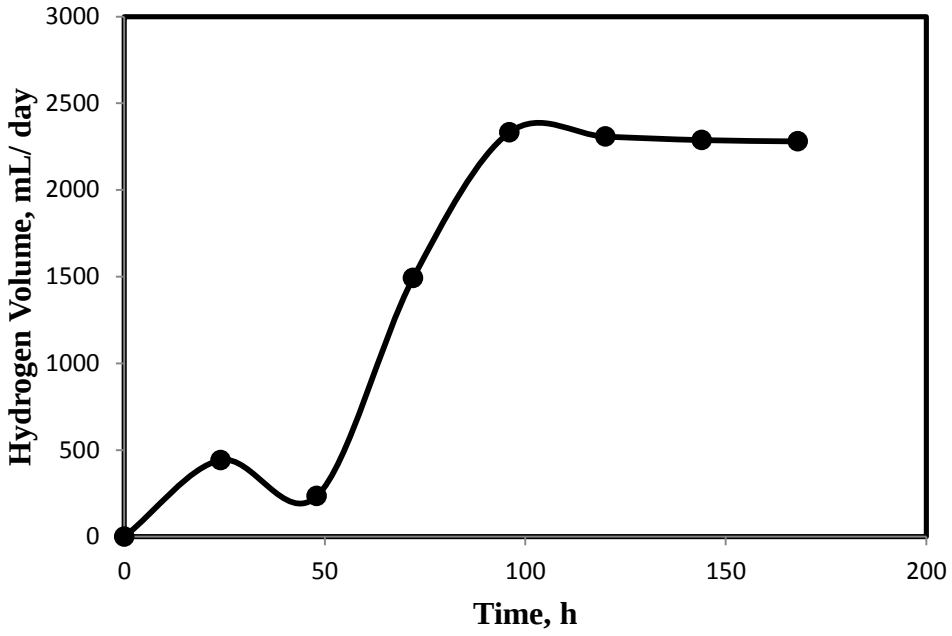


Figure 3.11: Variation of hydrogen gas volume with time at HRT=4.5 h

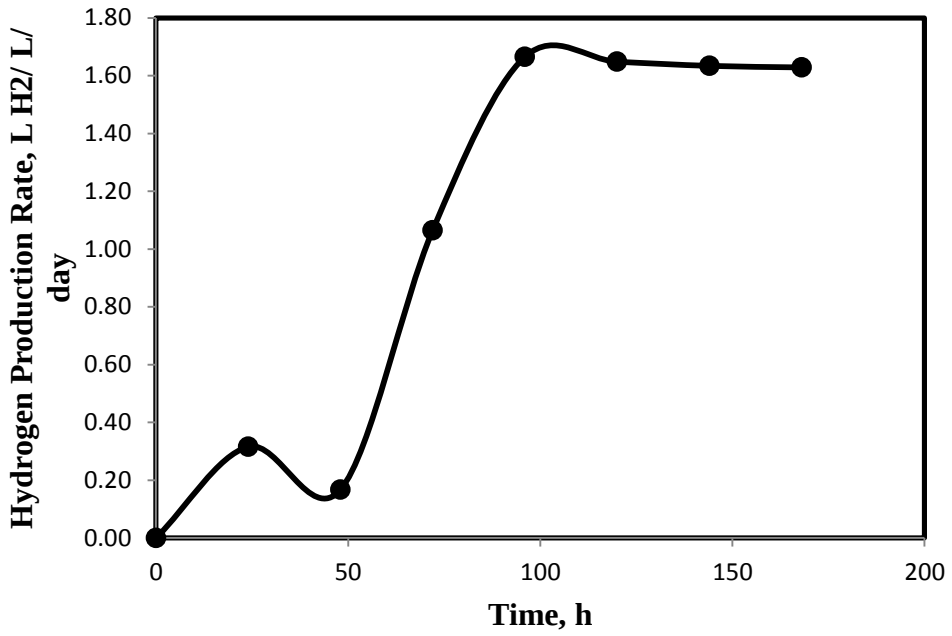


Figure 3.12 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at HRT =4.5 h

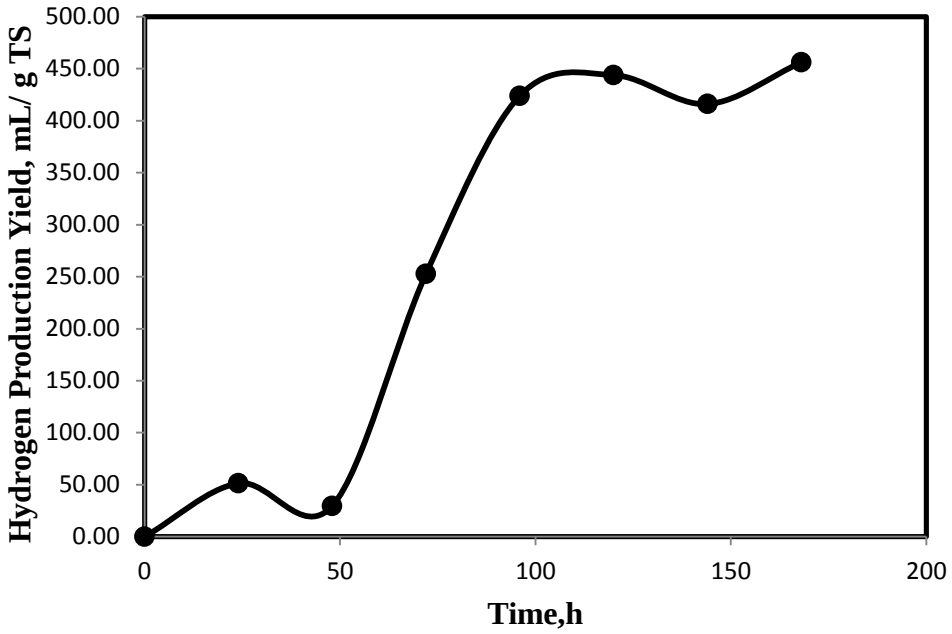


Figure 3.13 Variation of hydrogen production yield (mL/ g TS) with time at HRT =4.5 h

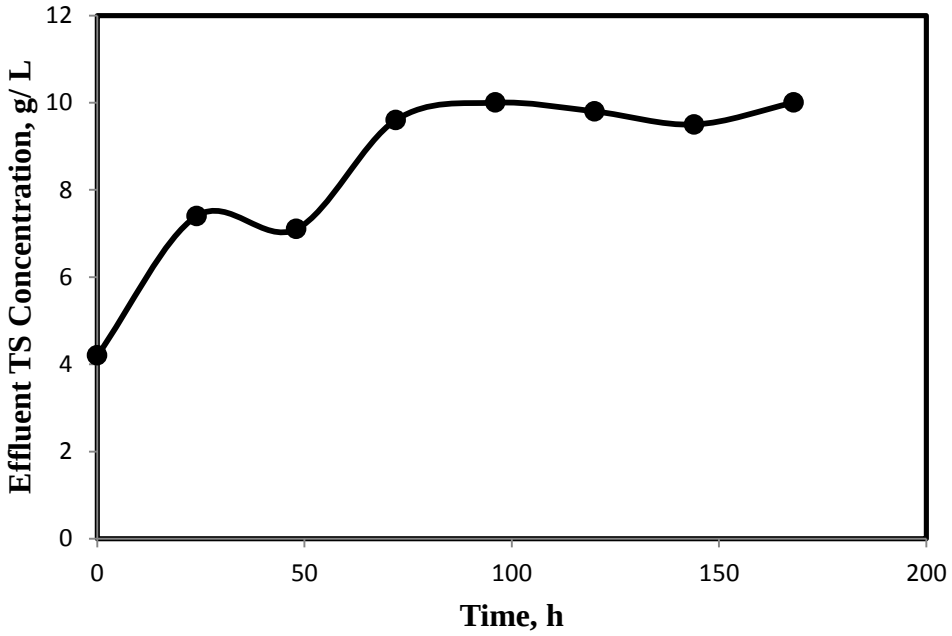


Figure 3.14 Variation of effluent total sugar concentration (g/ L) with time at HRT =4.5 h

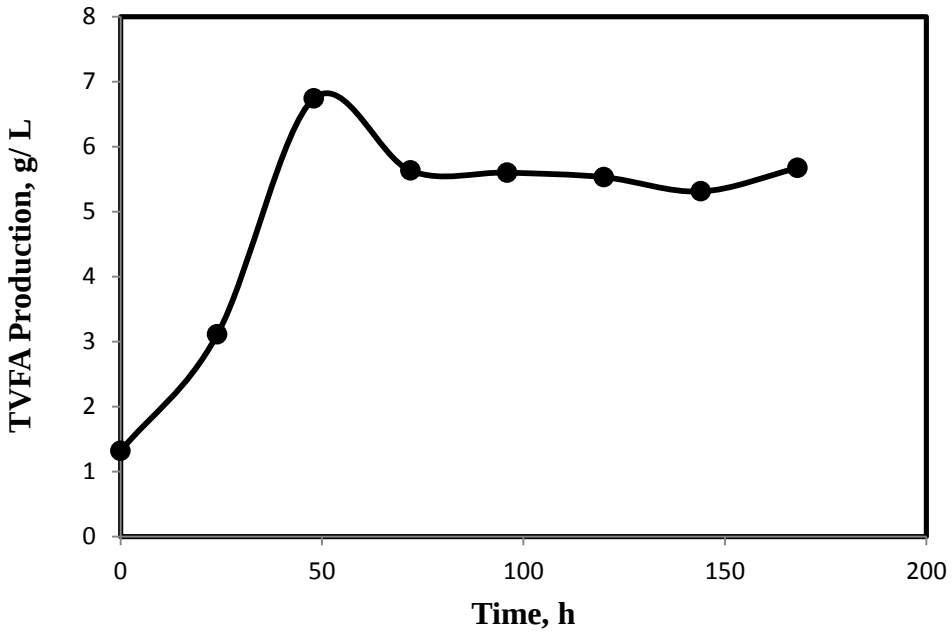


Figure 3.15 Variation of total volatile acids production (g/ L) with time at HRT =4.5 h

3.1.1.4 Hydrogen Production at HRT=3 h

Since reducing HRT from 8 h to 4.5 h resulted in improvement in overall performance of the system, the process was operated at HRT = 3 h in order to observe if further improvement in the production rate and yield can be achieved. Figure 3.16 depicts the time coarse of hydrogen volume at HRT = 3 h. The system reached to steady state within 30 hours. Shorter HRT substantially reduced total time required to reach the steady state. During the experimental studies at this HRT, the volume was monitored hourly and total gas volume production was evaluated daily. The total gas produced within 24 hour was 6300 mL which was the highest volume observed so far in the study. The percentage of hydrogen in the total gas volume was 50 ± 1 %. As a result, daily hydrogen production reached to 3519 mL/ day which is 2 times of the volume obtained at HRT = 4.5 h. The increase in the hydrogen volume at this HRT is almost 4 times with respect to HRT = 8 h and HRT = 6 h. As it was discussed in HRT = 4.5 h. High rate of substrate loading, increased the rate of hydrogen production and linear velocity to provide better separation of hydrogen bubbles from liquid phase to the head space of the reactor.

Decreasing HRT provided further improvement in production rate. Figure 3.17 presents the variation of volumetric hydrogen production rate with time. Maximum hydrogen production rate was obtained as 2.51 L H_2/ L/ day and hydrogen production yield was 645.69 mL/ g TS which was the highest obtained in continuous operation as shown in Figure 3.18. The improvements in the system performance with respect to HRT = 4.5 h were 1.5 times in the rate and 1.4 times in the yield.

Effluent total sugar concentration decreased from 15 g/ L feed concentration to nearly 9.5 g/ L yielding 37 % total sugar fermentation at HRT = 3 h (Figure 3.19). Produced TVFA concentration was approximately 3 g/ L was shown in Figure 3.20.

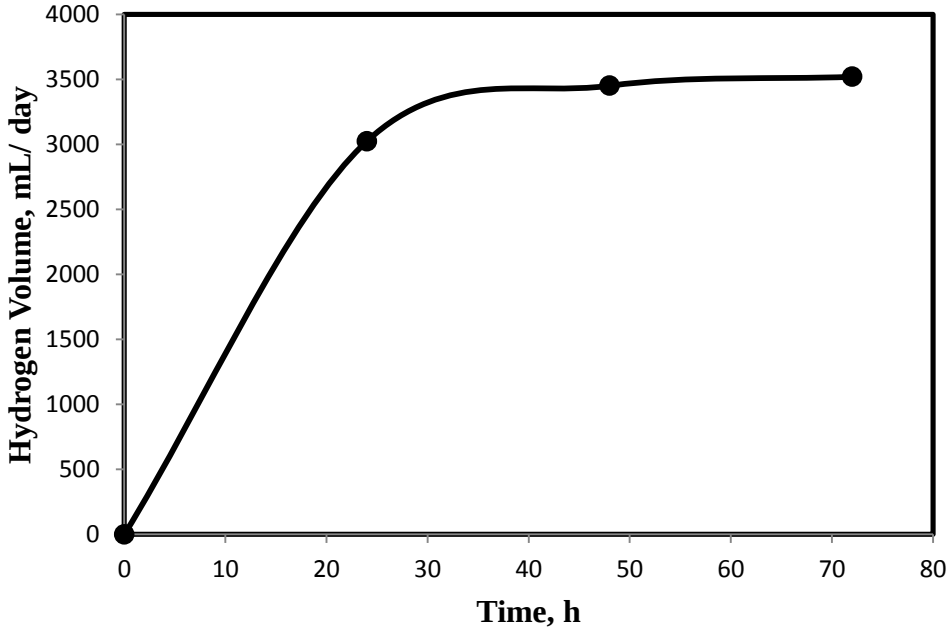


Figure 3.16 Variation of hydrogen gas volume with time at HRT=3 h

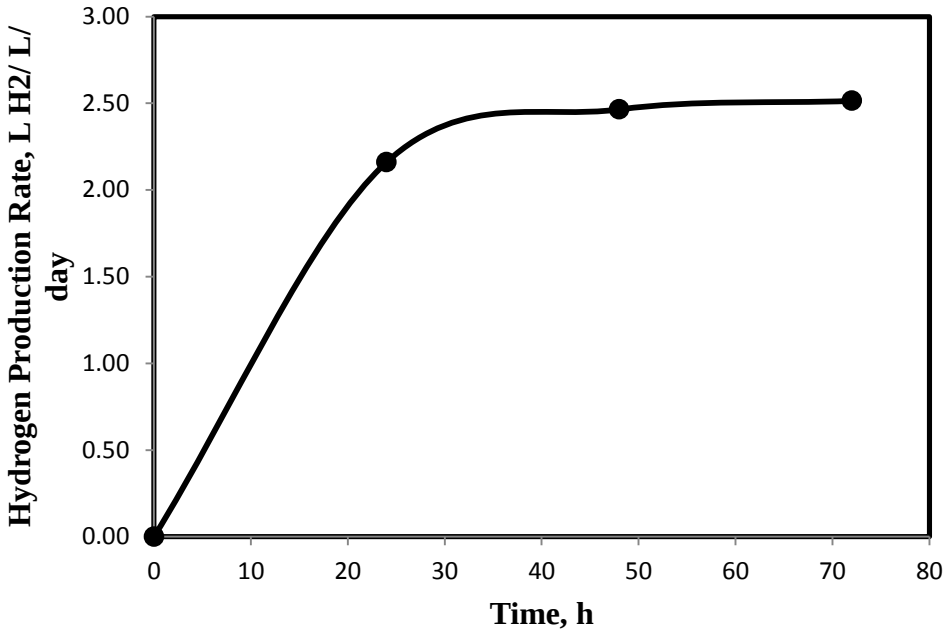


Figure 3.17 Variation of volumetric hydrogen production rate (L H_2/L/day) with time at HRT =3 h

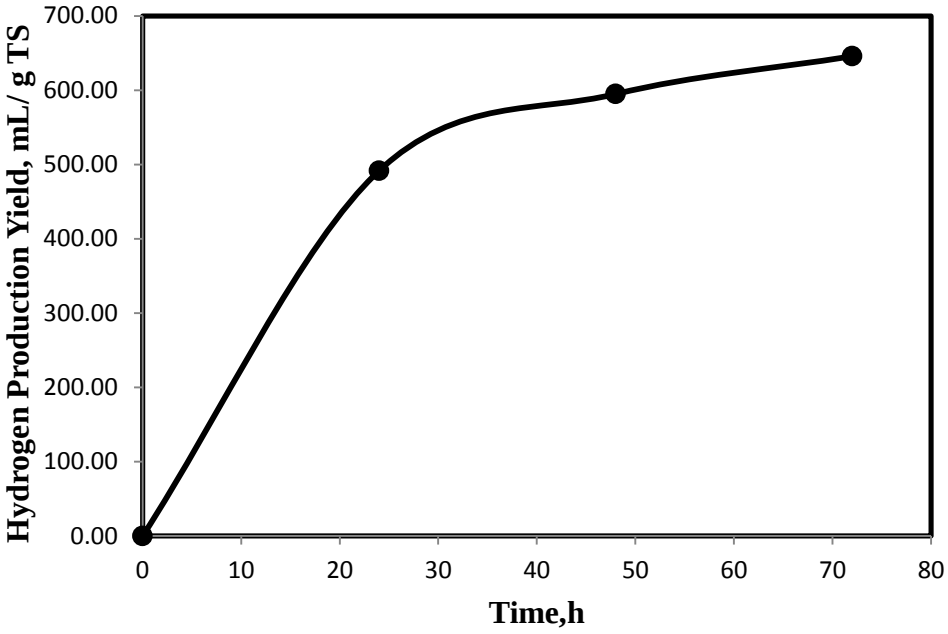


Figure 3.18 Variation of hydrogen production yield (mL/ g TS) with time at HRT =3 h

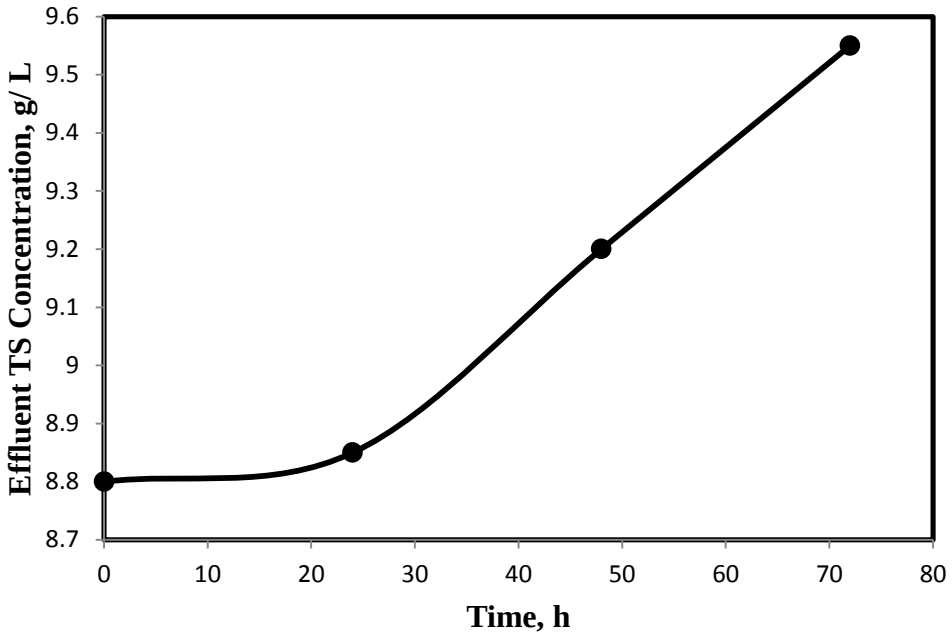


Figure 3.19 Variation of effluent total sugar concentration (g/ L) with time at HRT =3 h

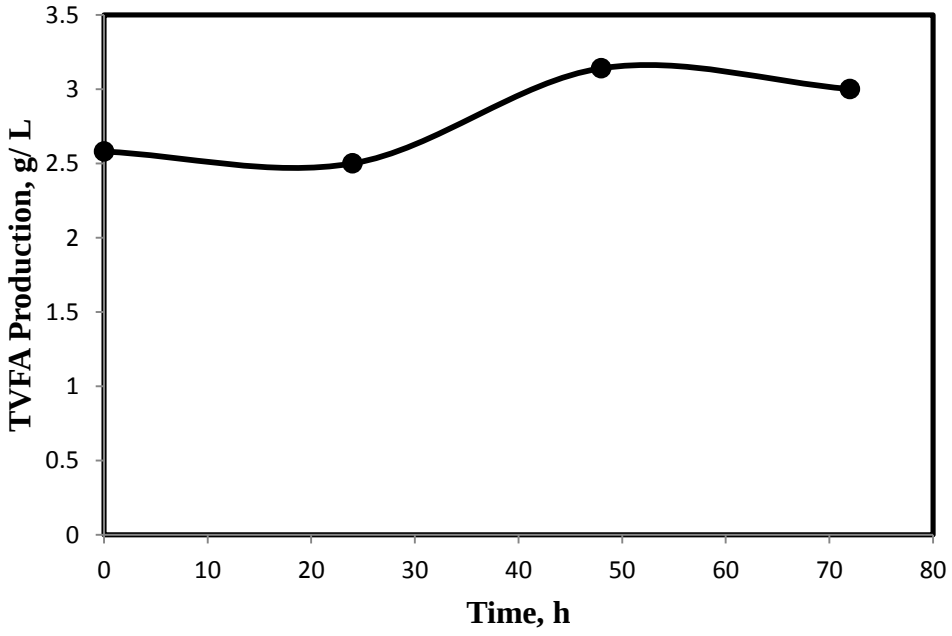


Figure 3.20 Variation of total volatile acids production (g/ L) with time at HRT =3 h

3.1.1.5 Hydrogen Production at HRT=2 h

The experimental studies revealed that decreasing HRT provided a considerable improvement in the hydrogen production. In biological processes which is operated for whether removal of substrate or generation of a product, a minimum hydraulic retention is determined. This parameter defines that it is the time at which microorganisms are washed out and almost no substrate removal or product formation occur. Washed out of organisms from the process means that growth rate of organisms is equal to dilution rate with feeding. Since microorganisms can't grow, they can't remove substrate and then no product formation. In order to determine minimum hydraulic retention time in up-flow anaerobic packed bed reactor with biological aquarium sponge support particle, it was aimed to run the system at lower hydraulic retention times as HRT = 2 h. The hydrogen volume obtained at this HRT was around 1961 mL/ day (Figure 3.21). About 2/3rd of hydrogen volume was obtained compared to the one observed at HRT = 3 h. So, there is a substantial decrease in the volume of production. This result show that the system is about the point of wash out of organisms. Hydrogen percentage in the steady state was 37 % which is again lower that percentage at HRT = 3 h. Similarly, the rate of formation was 1.40 L H_2/ L/ day (Figure 3.22) and yield of formation was 200 mL/ g TS (Figure 3.23). Effluent sugar concentration was about 5 g/ L (Figure 3.24) with percent removal of 67 % and finally effluent TVFA concentration varied between 1.78 g/ L to 3.22 g/ L (Figure 3.24). All these results indicate that the system is about the point of wash out of organisms when operated at HRT = 2 h.

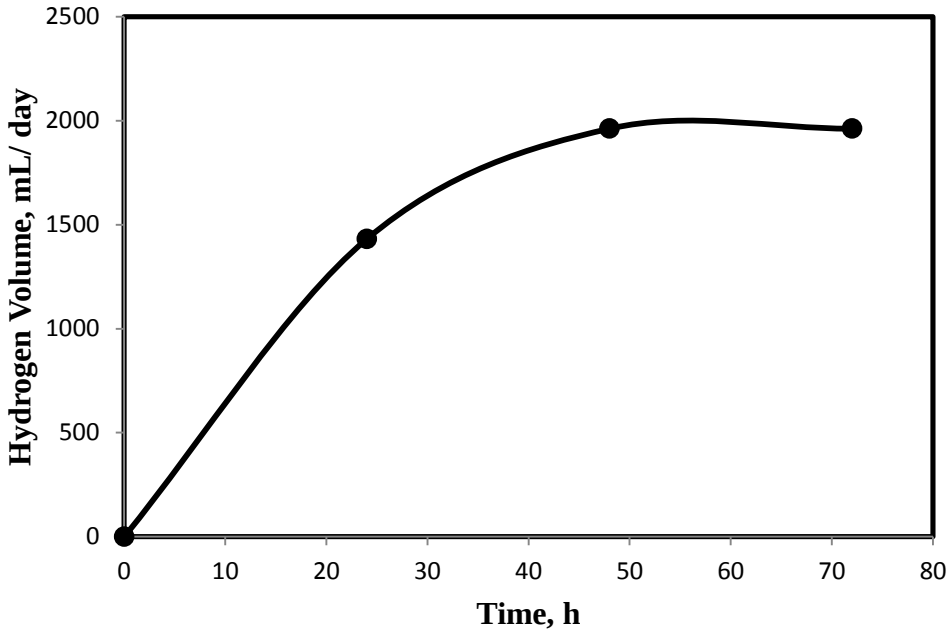


Figure 3.21 Variation of hydrogen gas volume with time at HRT=2 h

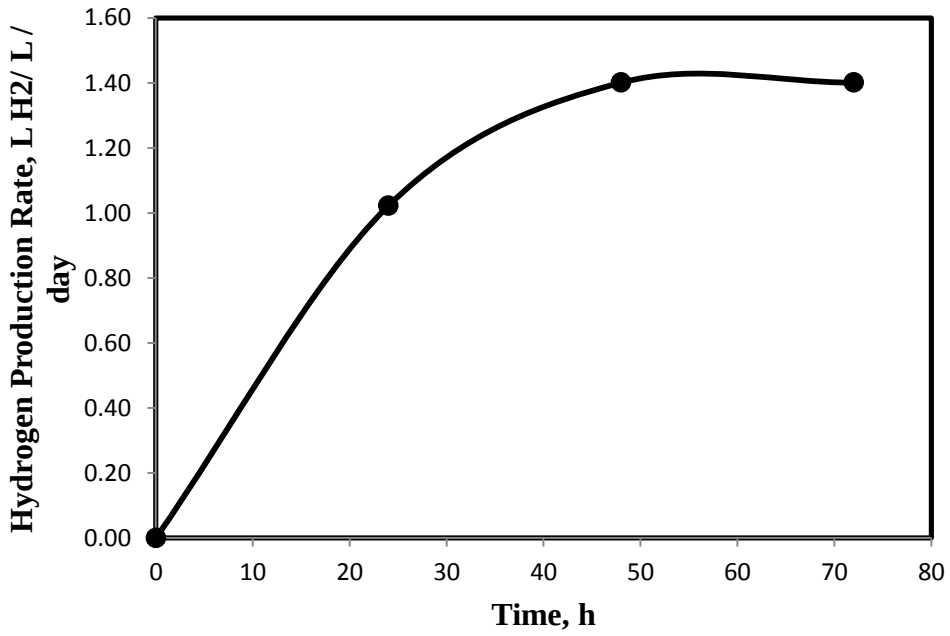


Figure 3.22 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at HRT =2 h

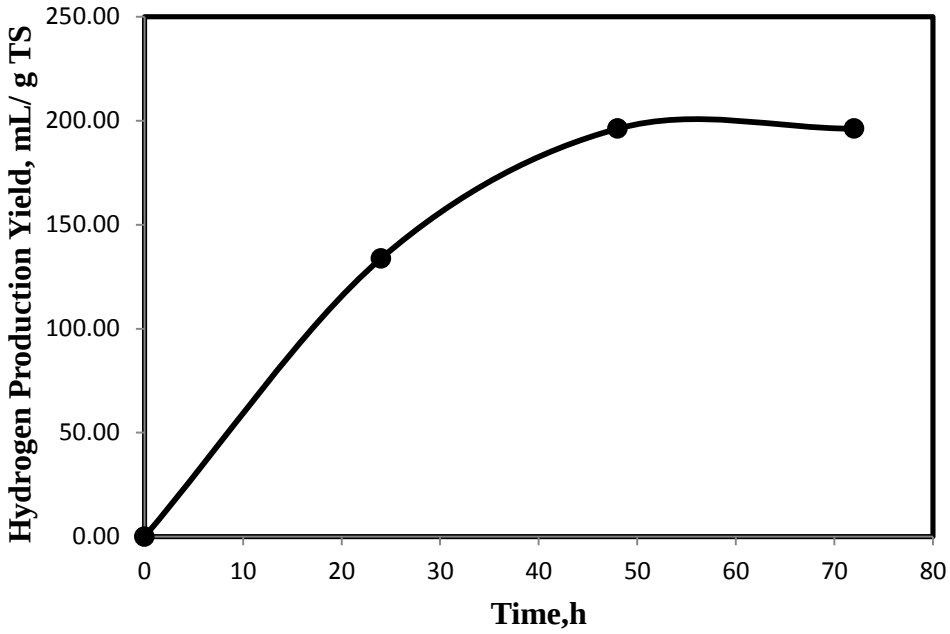


Figure 3.23 Variation of hydrogen production yield (mL/ g TS) with time at HRT =2 h

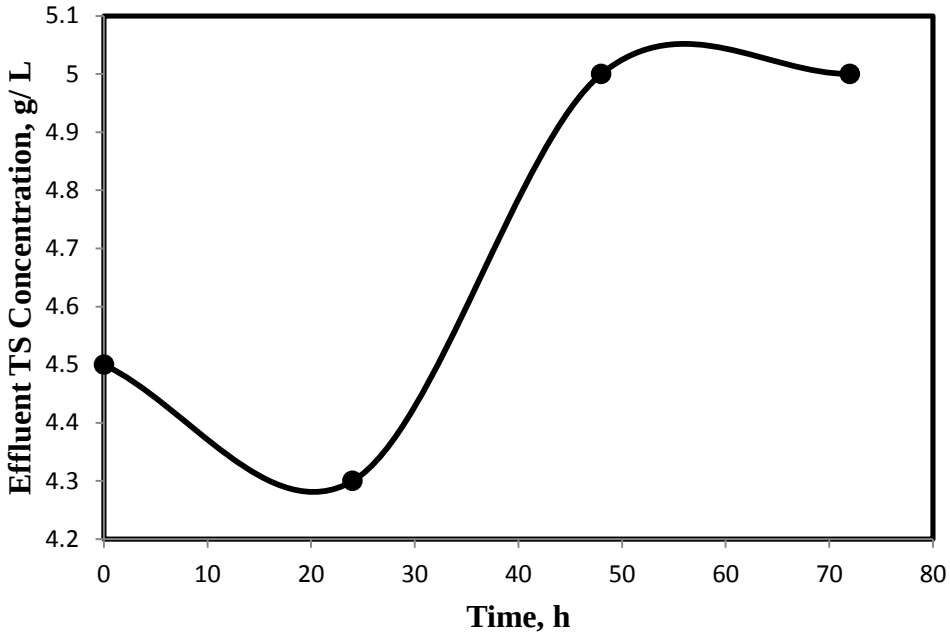


Figure 3.24 Variation of effluent total sugar concentration (g/ L) with time at HRT =2 h

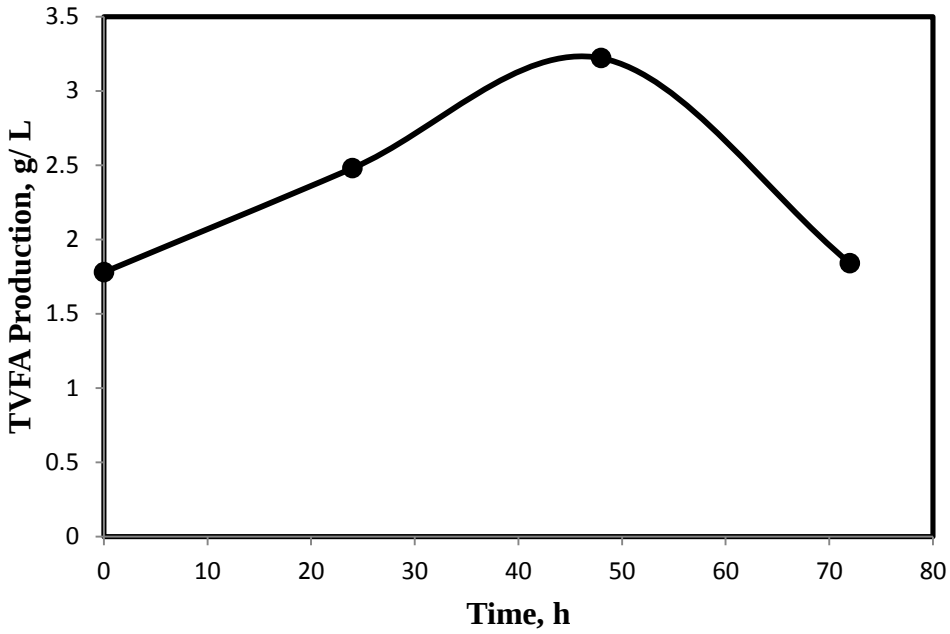


Figure 3.25 Variation of total volatile acids production (g/ L) with time at HRT =2 h

3.1.1.6 Hydrogen Production at HRT=1 h

Finally, reactor was operated at HRT=1 h which states very high rate of feeding and substrate loading. Variations of hydrogen gas volume with time at HRT=1 h is depicted in Figure 3.26. The production volume profile was quite different than the previous ones. The volume of hydrogen production was around 2000 mL for the first 20 hour and the sharply decreased to 1000 mL at which remained the same for the rest of the operation. The amount of hydrogen in the total gas was 15%. Maximum hydrogen production rate was 0.65 L H_2/ L/ day (Figure 3.27). In the first 24 h, yield of production was 223.13 mL/ g TS then a decreasing trend in the yield was observed. The final value was 116.35 mL/ g TS (Figure 3.28) for the time period between 48 h-72 h which corresponds to steady-state conditions with regard to hydrogen volume. About 50 % of total sugar was consumed by hydrogen producing bacteria. Effluent glucose concentration decreased from 12 g/ L to 8.2 g/ L in 72 h (Figure 3.29). TVFA was also produced in parallel with sugar consumption. The produced TVFA concentration was nearly constant at 2.4 g/ L in steady-state conditions as shown in Figure 3.30. These results indicate that system performance in terms of hydrogen production substantially decreased mainly due to wash out of organisms form the reactor. The other factor is the high rate of substrate loading

which could cause substrate inhibition. In other words, the system is operated at very high substrate loading rates, but there is no enough biomass to process the substrate. The organisms are washed out from the system with the effect of shear force during high linear velocity at low HRTs or high feeding rates.

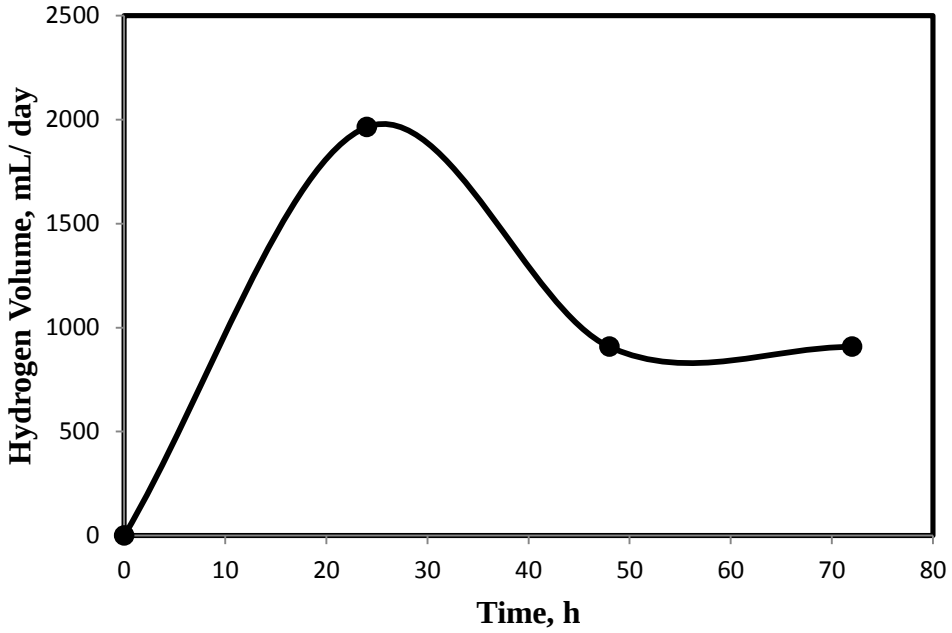


Figure 3.26 Variation of hydrogen gas volume with time at HRT=1 h

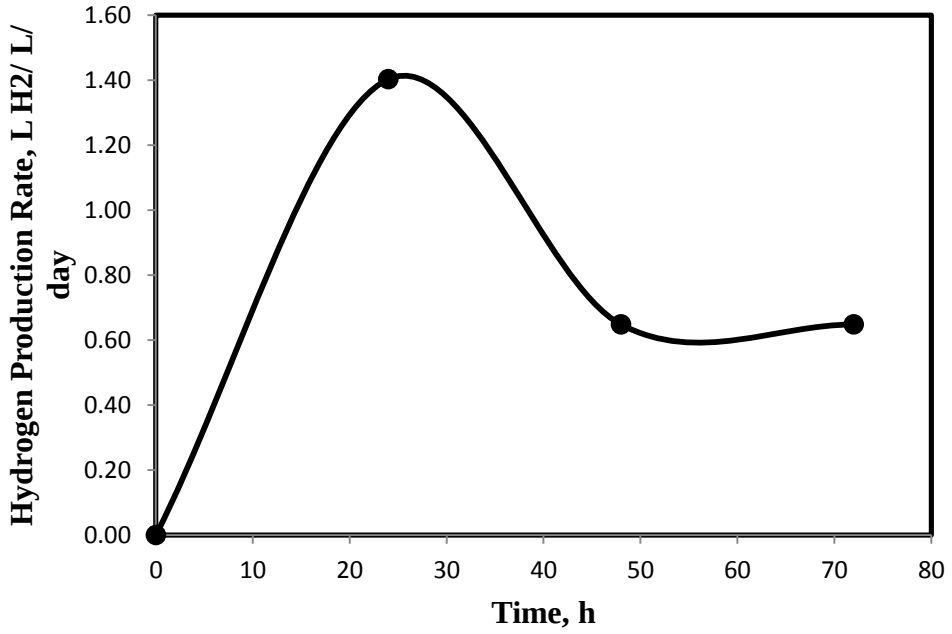


Figure 3.27 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at HRT =1 h

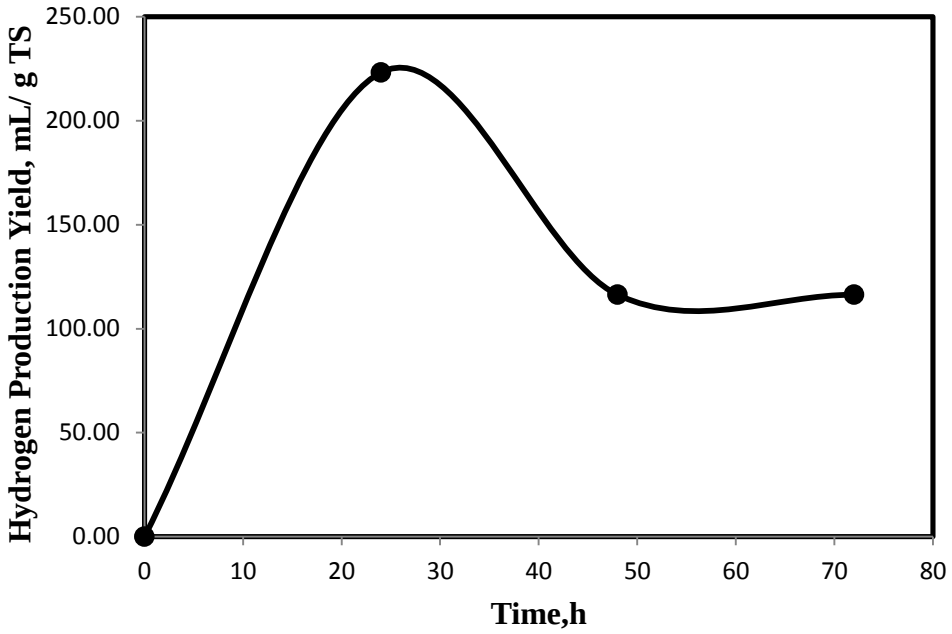


Figure 3.28 Variation of hydrogen production yield (mL/ g TS) with time at HRT =1 h

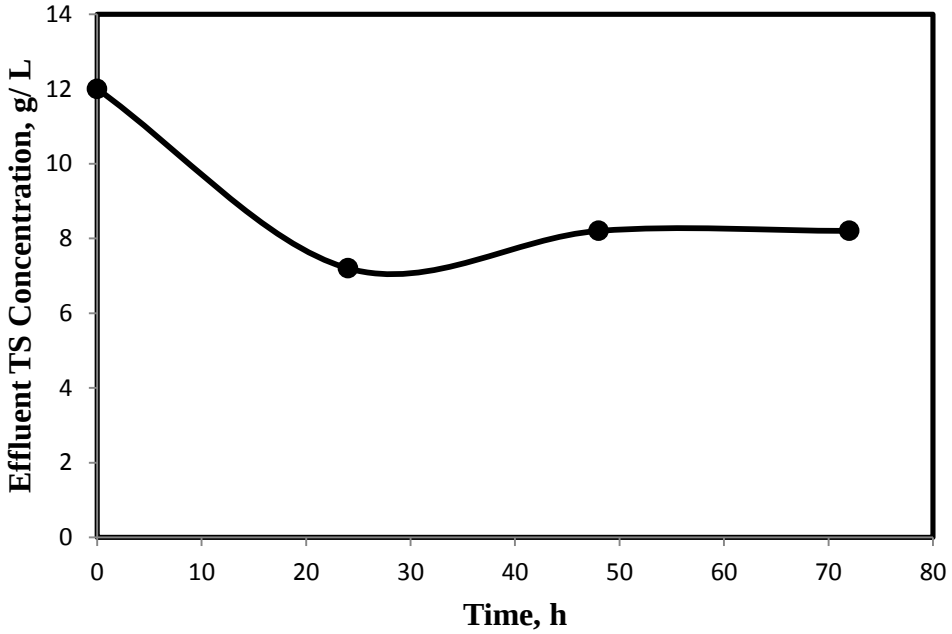


Figure 3.29 Variation of effluent total sugar concentration (g/ L) with time at HRT =1 h

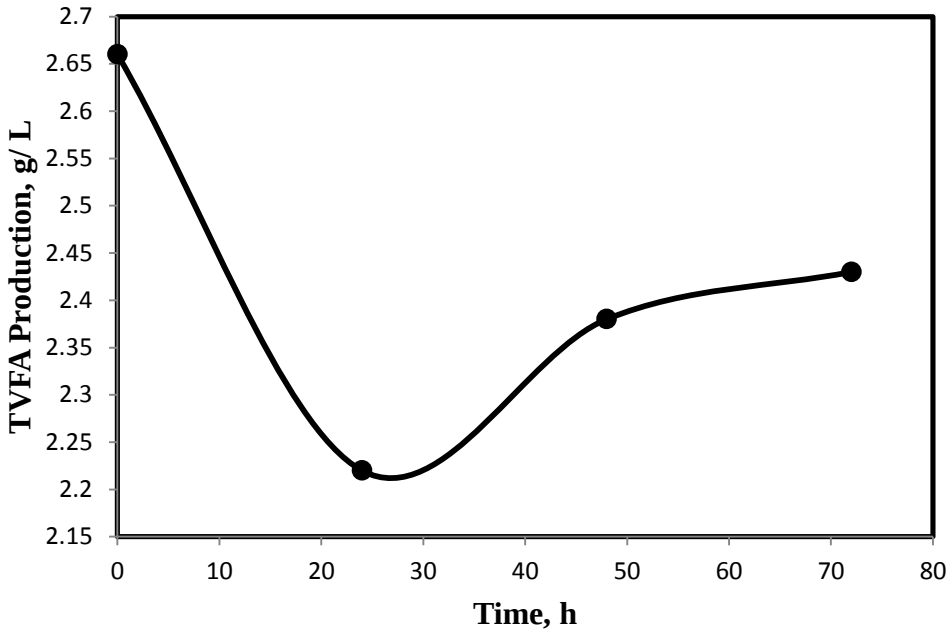


Figure 3.30 Variation of total volatile acids production (g/ L) with time at HRT =1 h

3.1.2 Effect of Initial Substrate Concentration on Hydrogen Production

Hydraulic retention time was one of the main parameter in evaluating the system performance. The other one is the initial substrate concentration. Therefore the system was operated at different initial substrate concentrations as TSo=10 g/ L, 15 g/ L, 19 g/ L, 25 g/ L, 35 g/ L. HRT was kept constant at HRT=3 h at which maximum volume, yield and rate of productions were achieved.

3.1.2.1 Hydrogen Production at 10 g/ L Initial Total Sugar

The system was first operated at low substrate concentration as TS_0= 10 g/ L. Daily hydrogen gas production volume was around 550 mL/ day, although daily total gas production volume was around 2500 mL/ day for the time period from 24 h till 72 h. The hydrogen percentage was nearly 22 % in the total gas. The results up to this point reveal that there is decrease in the system performance compared to the results obtained at 15 g/ L substrate concentration and HRT=3 h. The steady state conditions were reached within 20 h which is the same as previous experiment. Maximum hydrogen production rate and yield with time at 10 g/ L initial sugar concentration were 0.41 L H_2/ L/ day and 684.52 mL/ g TS, respectively (Figure 3.32 and Figure 3.33). It is obvious that there was substrate limitation under these

operation conditions. Substrate utilization was around 10 % with effluent sugar concentration of 9 g/ L (Figure 3.34). Since about 1 g/ L substrate is used, it is acceptable to obtain low TVFA concentration in the effluent as 0.26 g/ L (Figure 3.35). In summary, 10 g/ L initial total sugar concentration was not sufficient to sustain the growth of the biomass and in turn hydrogen production. On the other hand, the reason why culture can't consume about 9 g/ L sugar is not clearly understood.

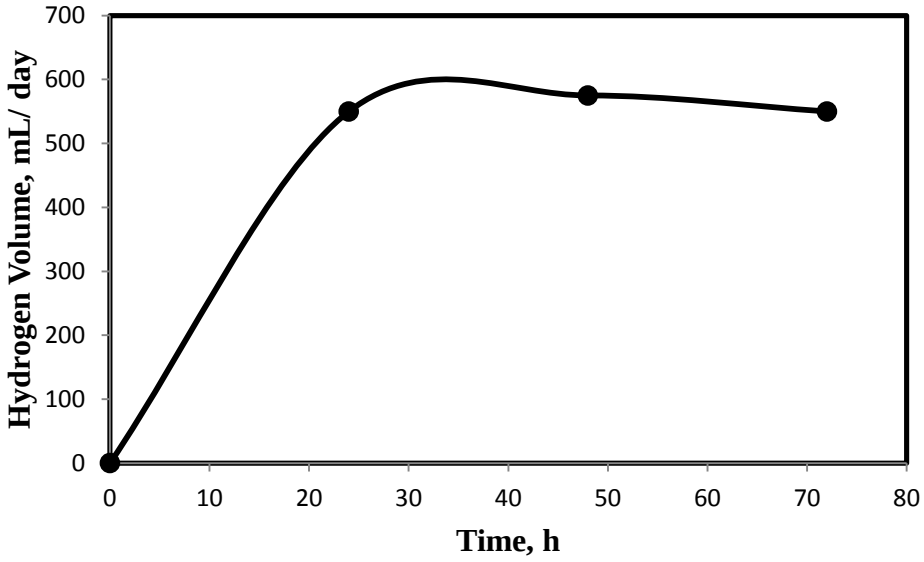


Figure 3.31 Variation of hydrogen gas volume with time at TSo=10 g/ L

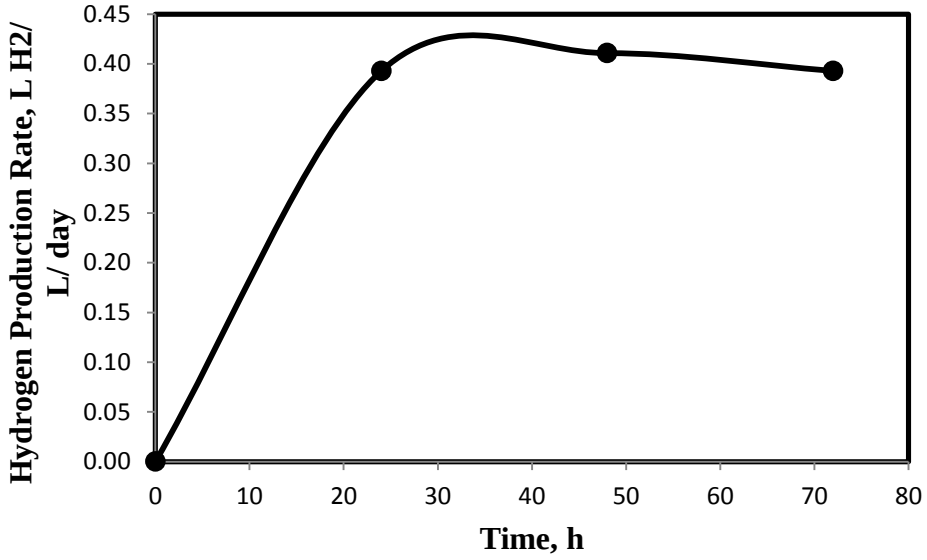


Figure 3.32 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at TSo=10 g/ L

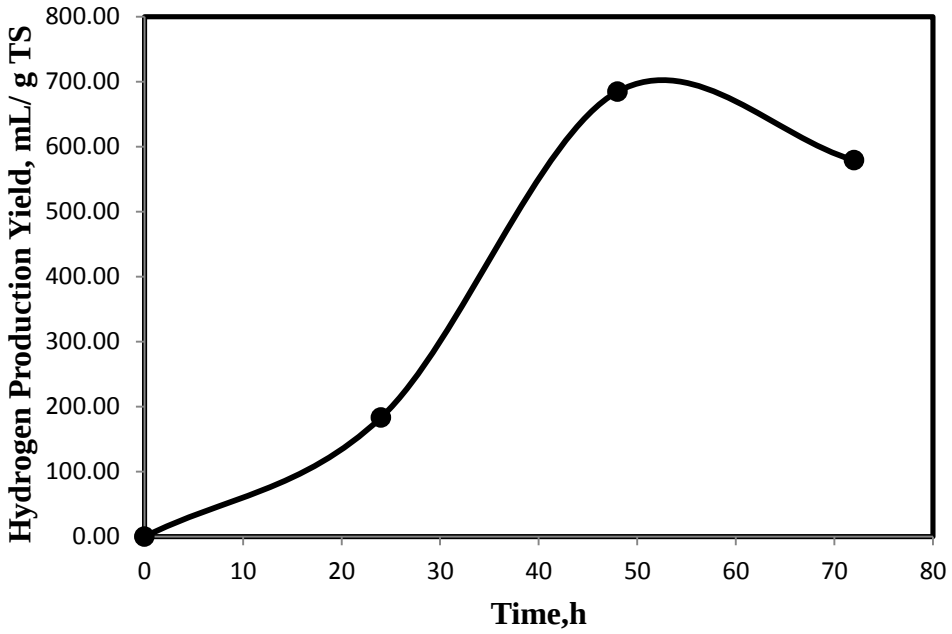


Figure 3.33 Variation of hydrogen production yield (mL/ g TS) with time at TSo=10 g/ L

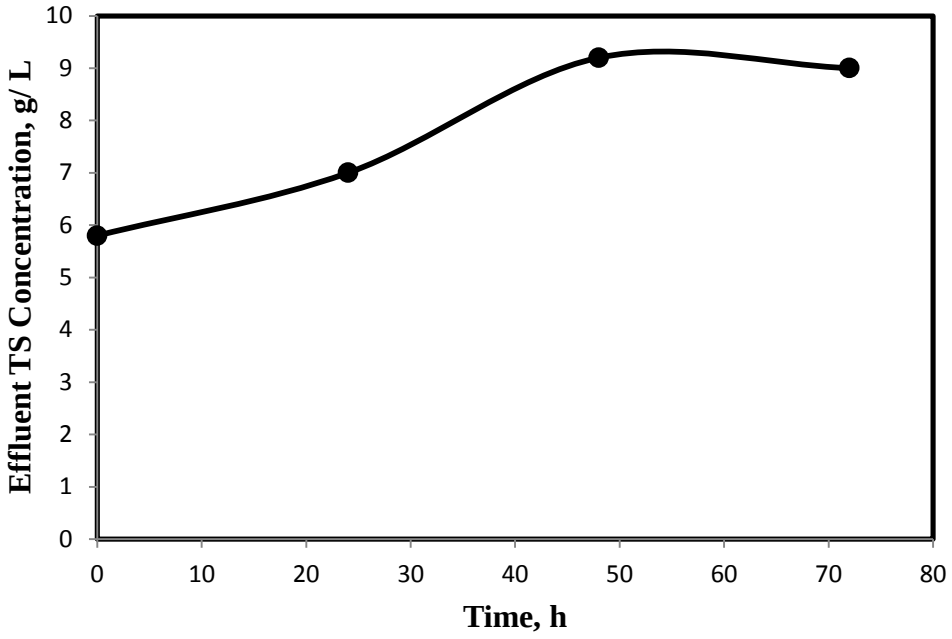


Figure 3.34 Variation of effluent total sugar concentration (g/ L) with time at TSo=10 g/ L

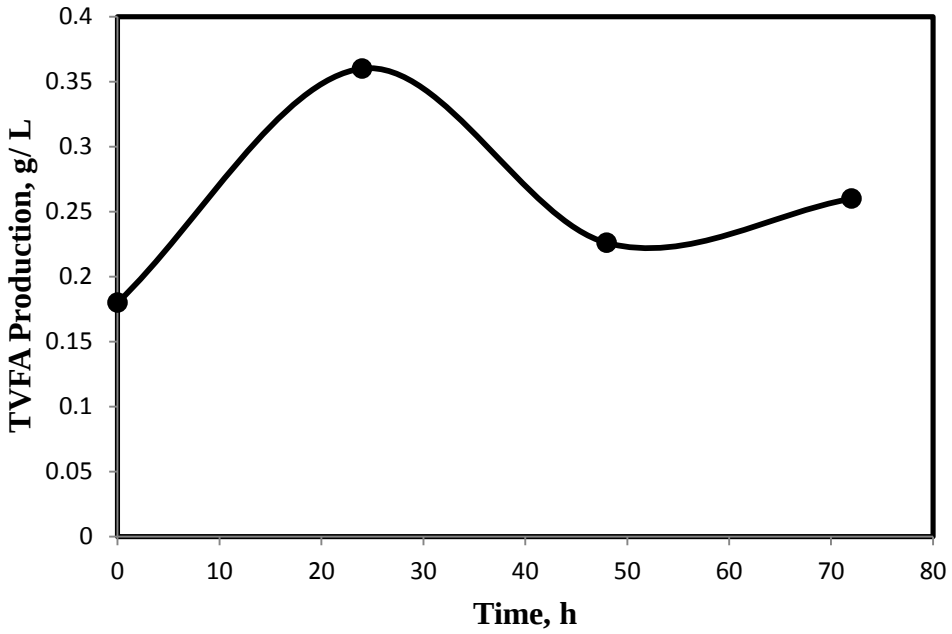


Figure 3.35 Variation of total volatile acids production (g/ L) with time at TSo=10 g/ L

3.1.2.2 Hydrogen Production at 15 g/ L Initial Total Sugar

Figure 3.36 depicts time course of variation of hydrogen gas volume at 15 g/ L initial sugar concentrations. The highest hydrogen volume was 3519 mL at the end of the fermentation. The percentage of hydrogen was measured 36 % ± 2 %. Maximum hydrogen production rate and yield were 2.51 L H_2/ L/ day and 645.69 mL/ g TS for the initial total sugar contents at 15 g/ L (Figure 3.37 and Figure 3.38, respectively). Effluent total sugar concentration remained almost constant around 9.2 ± 0.35 g/ L for the last 2 days with average 36 % removal efficiency (Figure 3.39). An increase in total volatile organic acids production from nearly 2.5 g/ L to 3 g/ L due to a good total sugar consumption, was observed (Figure 3.40).

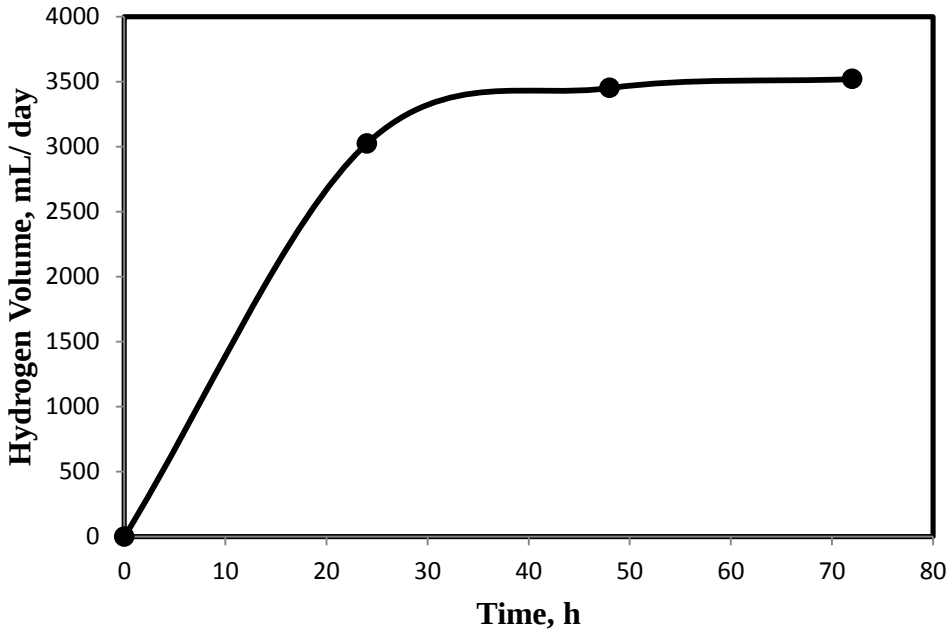


Figure 3.36 Variation of hydrogen gas volume with time at TSo=15 g/ L

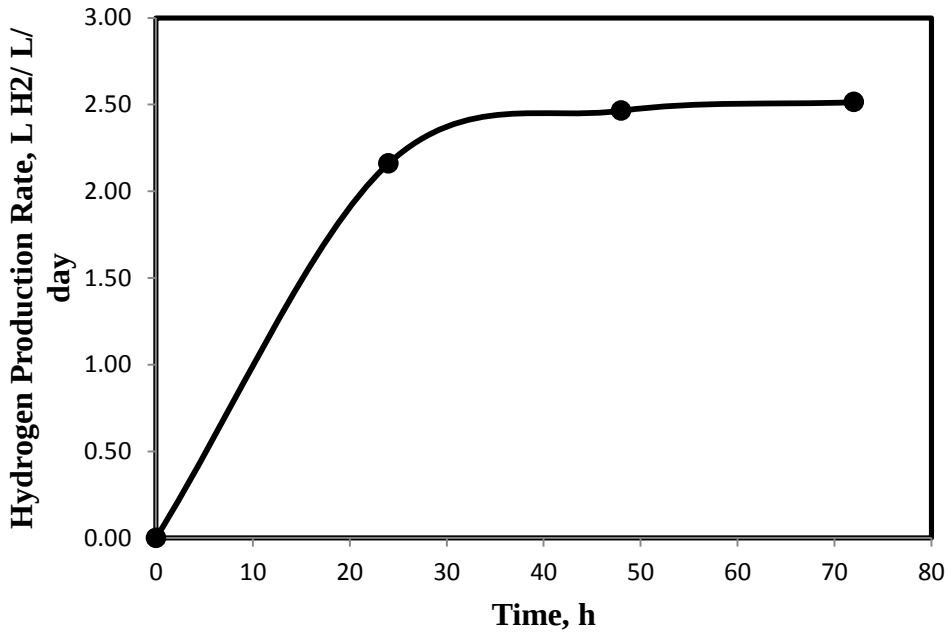


Figure 3.37 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at TSo=15 g/ L

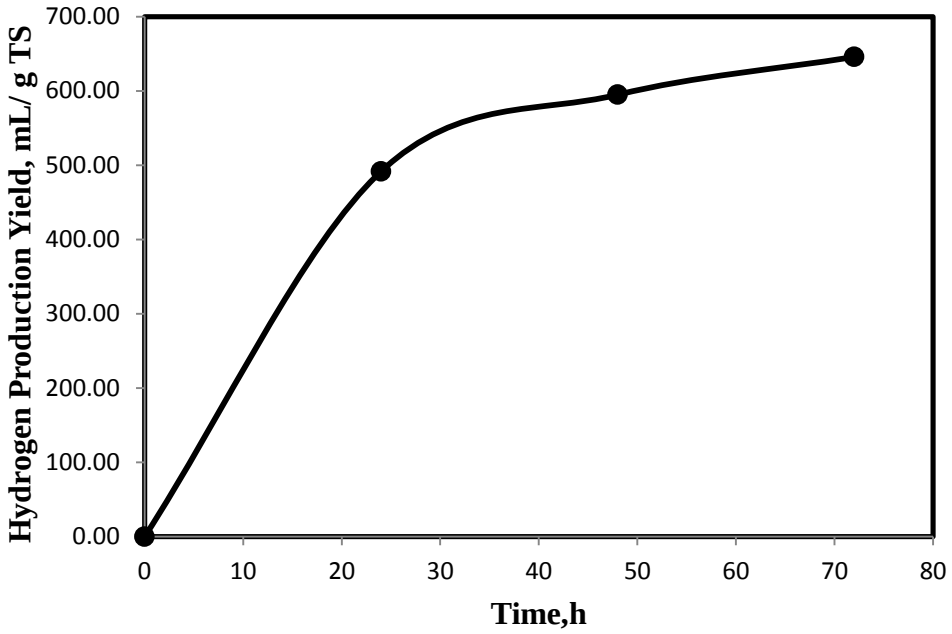


Figure 3.38 Variation of hydrogen production yield (mL/ g TS) with time at TSo=15 g/ L

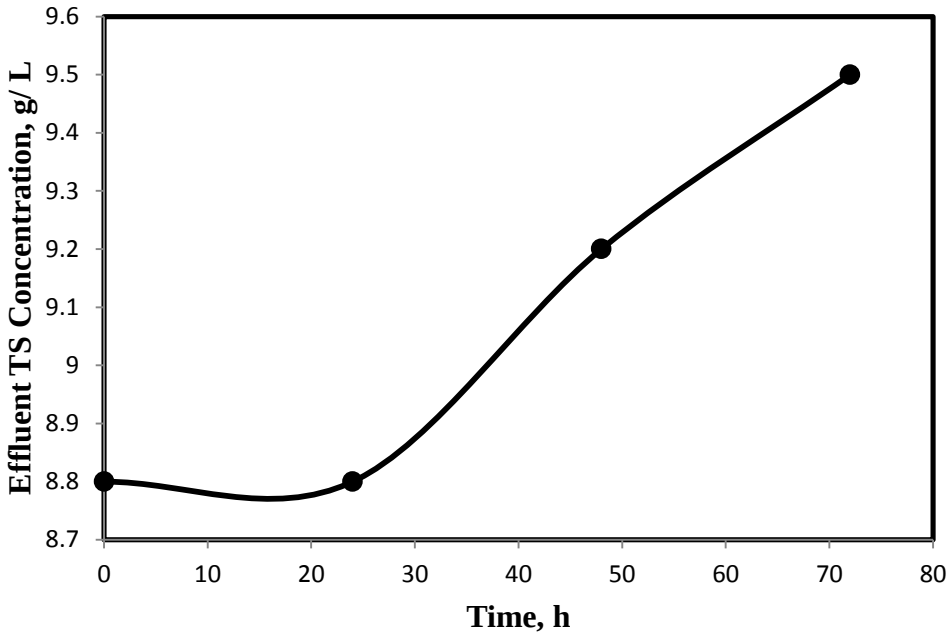


Figure 3.39 Variation of effluent total sugar concentration (g/ L) with time at TSo=15 g/ L

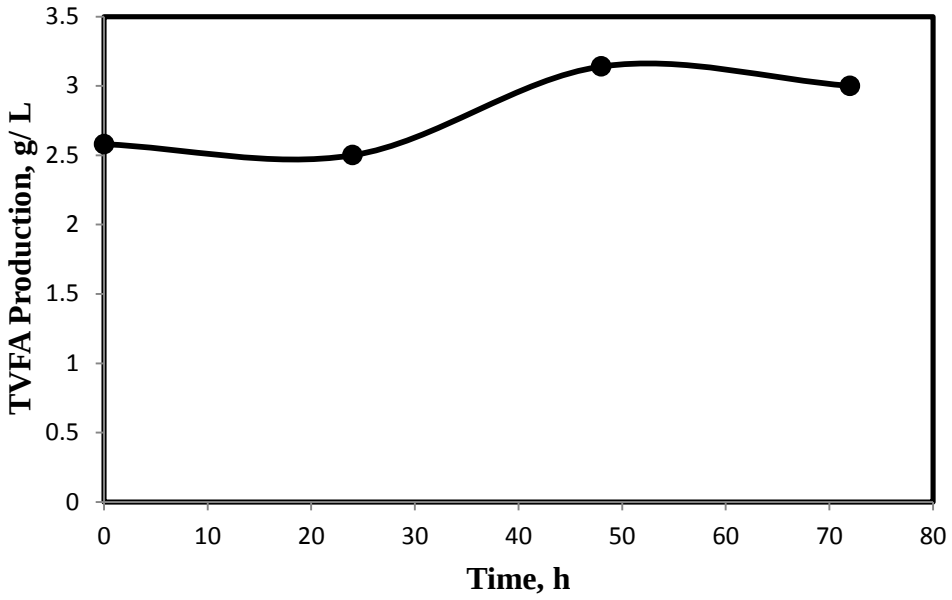


Figure 3.40 Variation of total volatile acids production (g/ L) with time at TSo=15 g/ L

3.1.2.3 Hydrogen Production at 19 g/ L Initial Total Sugar

Figure 3.41 shows variation of hydrogen production volume with time at 19 g/ L initial total sugar concentration. Daily total gas production was 4050 mL in the first 24 h of the fermentation. After 24 h, system reached to steady-state conditions and daily hydrogen production volume was obtained as 3876 mL/ day. The hydrogen percentage was 50 % ± 1 % at steady-state condition. Hydrogen production rate was 2.77 L H_2/ L/ day (Figure 3.42). Yield of production was 206 and 428 mL/ g TS for the first and second day, respectively. Then, an increasing trend in the yield was observed and it reached to maximum level of 1938 mL/ g TS for the last 48 h (Figure 3.43). A very low sugar consumption was observed. Effluent sugar concentration was around 17 g/ L with 11 % removal at the steady-state conditions as given in Figure 3.44. Produced TVFA concentration was nearly 3.8 g/ L for the last 2 days (Figure 3.44). The rate was 2.51 L H_2/ L/ day at TS_o= 15 g/ L and rised to 2.77 L H_2/ L/ day at TS_o= 19 g/ L without substantial improvement in the rate. However, increasing substrate concentration from 15 g/ L to 19 g/ L provided an substantial improvement in the yield of production. It was 645.69 mL/ g TS at TS_o= 15 g/ L, and rised to 1938 mL/ g TS for TS_o= 19 g/ L. One of the other finding in this set of experiment is higher TVFA production which enhances hydrogen generation.

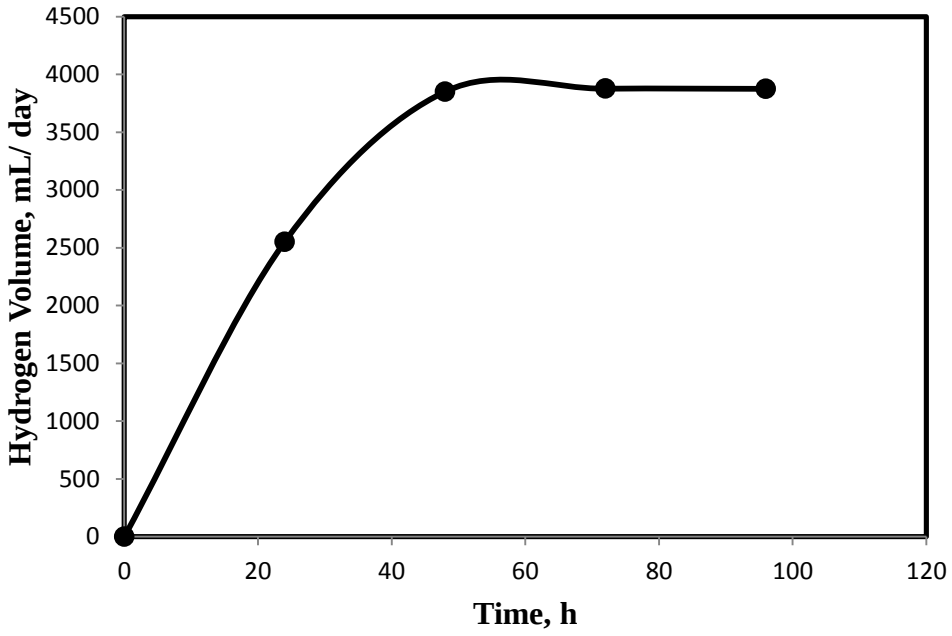


Figure 3.41 Variation of hydrogen gas volume with time at TSo=19 g/ L

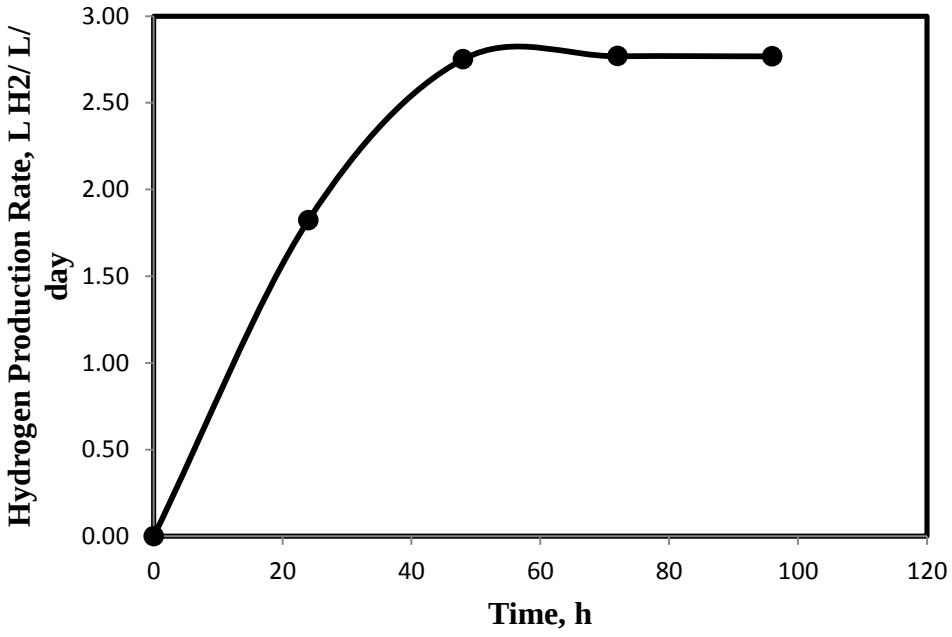


Figure 3.42 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at TSo=19 g/ L

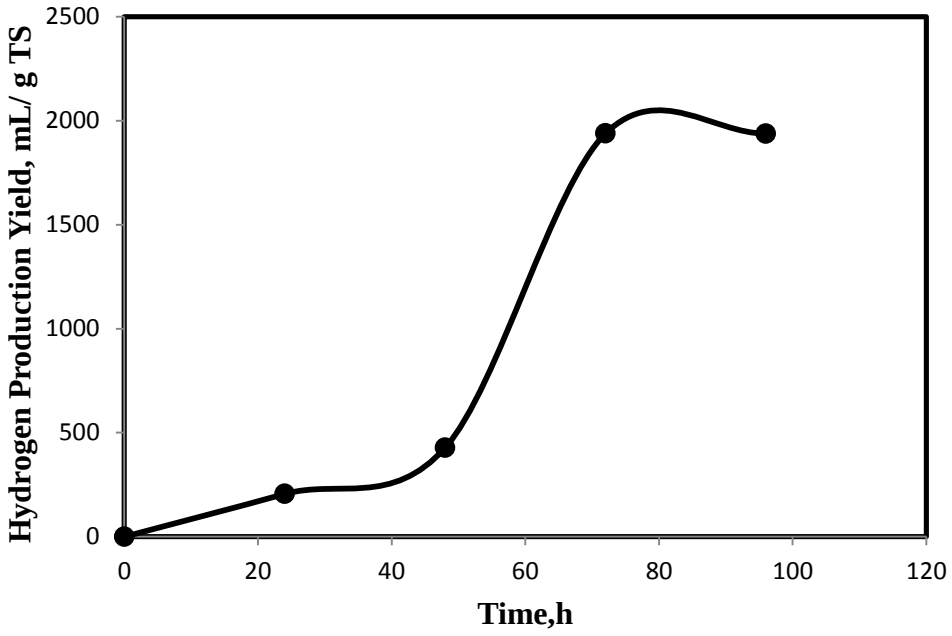


Figure 3.43 Variation of hydrogen production yield (mL/ g TS) with time at TSo=19 g/ L

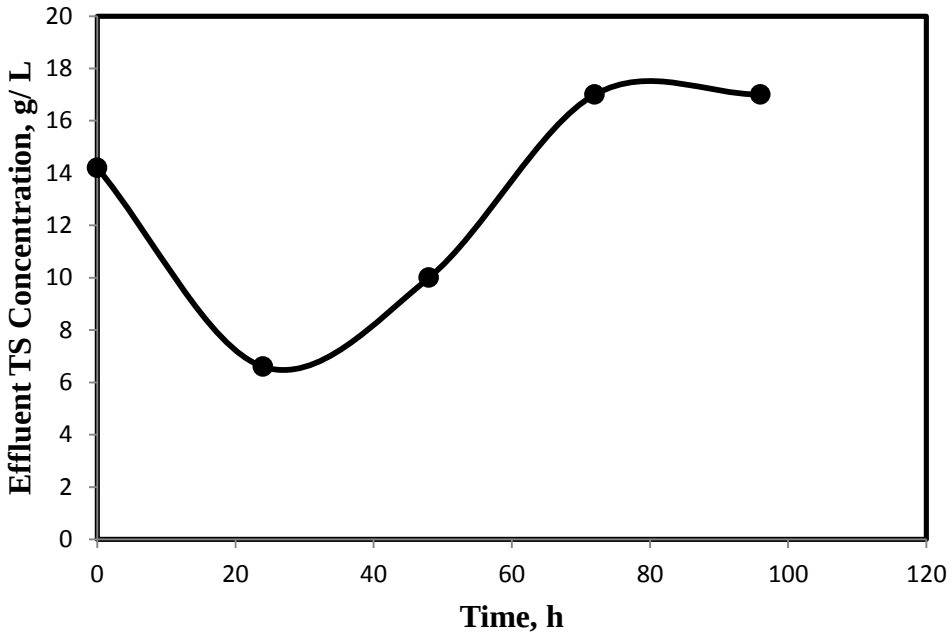


Figure 3.44 Variation of effluent total sugar concentration (g/ L) with time at TSo=19 g/ L

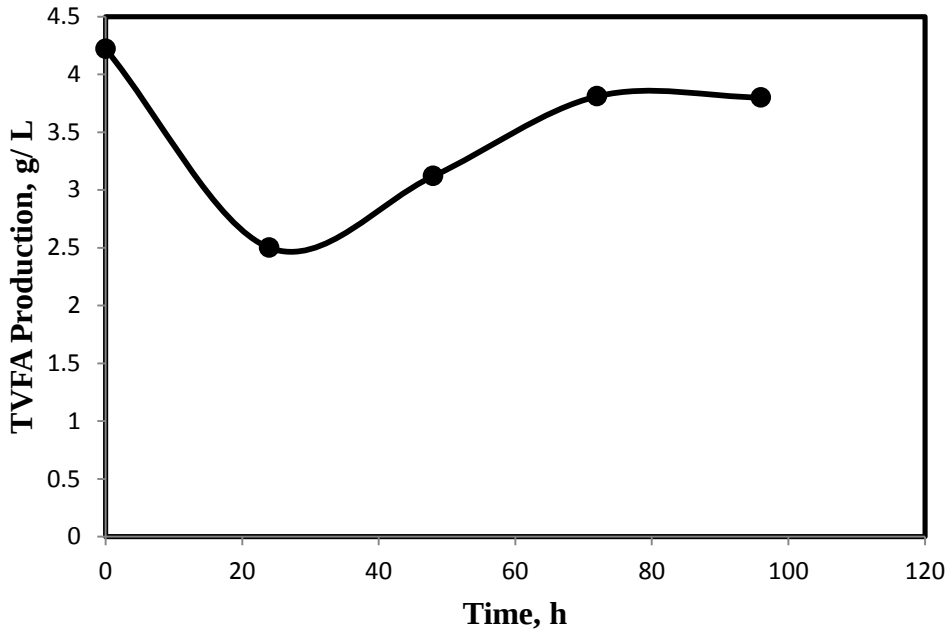


Figure 3.45 Variation of total volatile acids production (g/ L) with time at TSo=19 g/ L

3.1.2.4 Hydrogen Production at 25 g/ L Initial Total Sugar

Increasing initial total sugar concentration at HRT=3 h operation provided an improvement in the hydrogen production potential of the process. Therefore, further increase in the concentration is performed and the process was operated at 25 g/ L initial substrate concentration. Figure 3.46 depicts time course of variation of hydrogen volume by dark fermentation when the initial total sugar concentration was 25 g/ L. Hydrogen volume was 1134 mL/ day at the beginning of operation and then a substantial increase in the volume to 4275 mL/ day was achieved. In addition, hydrogen percentage in the total gas reached to the level of 82 % ± 1 %. The maximum production rate (3.05 L H_2/ L/ day) was also obtained at this concentration (Figure 3.47). These were the maximum hydrogen production performance values so far obtained in the contents of the study. Nevertheless, yield of production was around 854.90 mL/ g TS (Figure 3.48) which is lower that one obtained at 19 g/ L initial substrate concentration and at HRT=3 h.

Figure 3.49 and Figure 3.50 depict variation of effluent total sugar and produced total volatile organic acid concentration with time course at 25 g/ L initial substrate concentration. The effluent total sugar was 20 g/ L in the first day and no substantial

change in the concentration was observed for further days. Nearly 20 % initial total sugar was consumed by microorganism throughout the experiment. In parallel to low substrate utilization, TVFA production was low (1 g/ L) as well. Although low TVFA formation and substrate utilization occurred in this experiment, yield and rate of production was considerably high. This results can be explained as that, the produced volatile fatty acids could be mainly acetic acid rather that butyric acid. Since, yield of hydrogen production from acetic acid is higher than that of butyric acid.

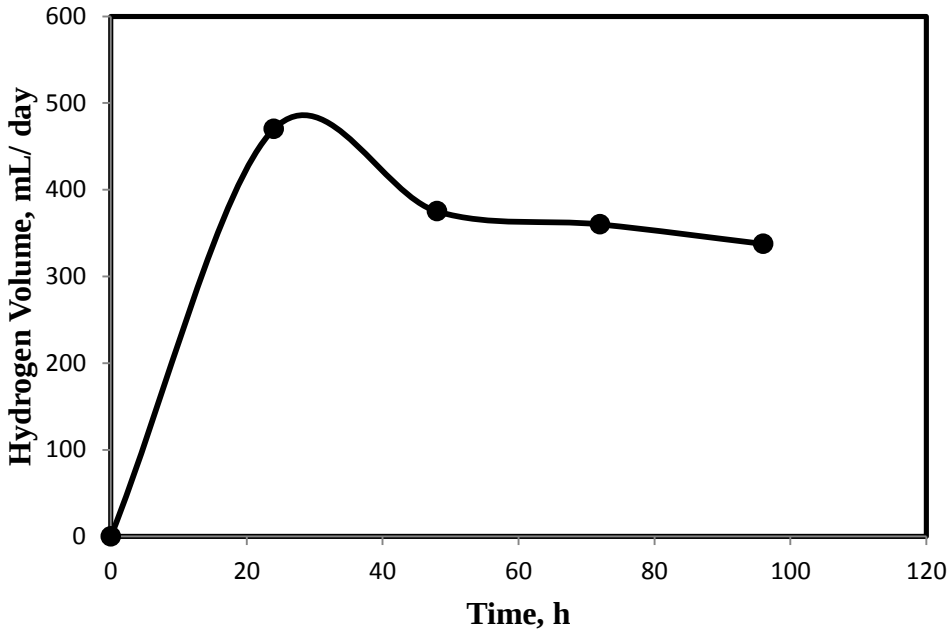


Figure 3.46 Variation of hydrogen gas volume with time at TSo=25 g/ L

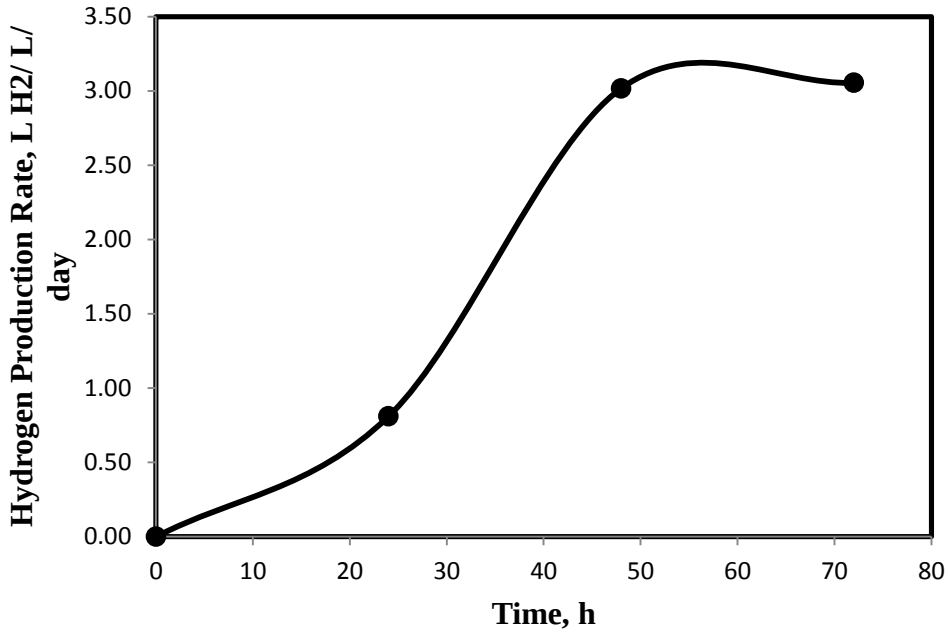


Figure 3.47 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at TSo=25 g/ L

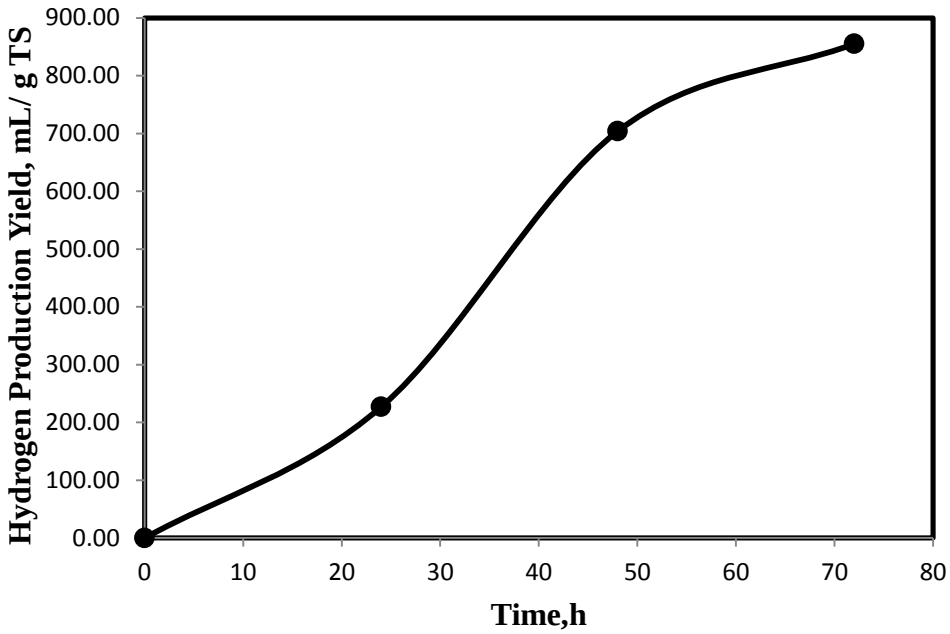


Figure 3.48 Variation of hydrogen production yield (mL/ g TS) with time at TSo=25 g/ L

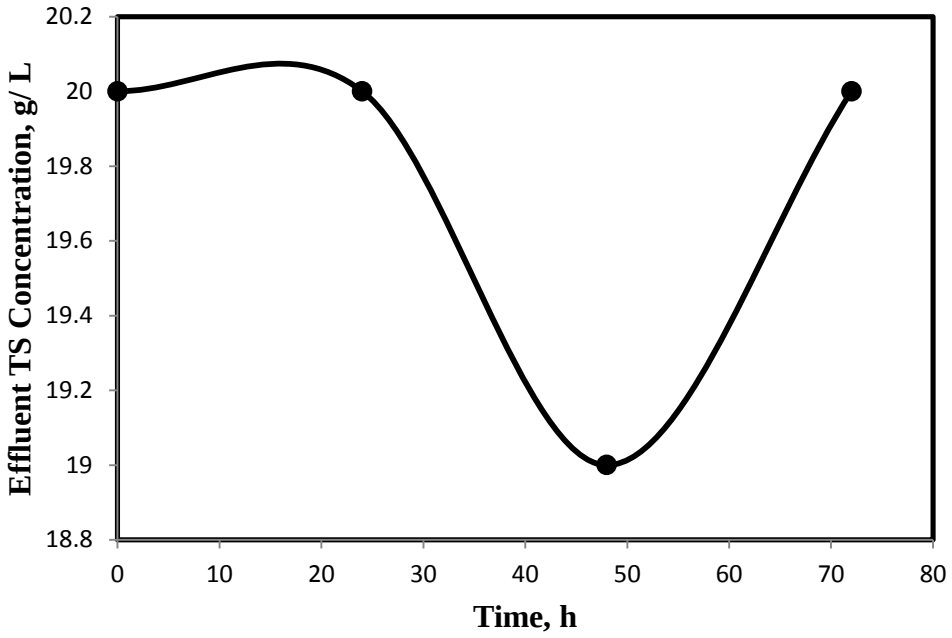


Figure 3.49 Variation of effluent total sugar concentration (g/ L) with time at TSo=25 g/ L

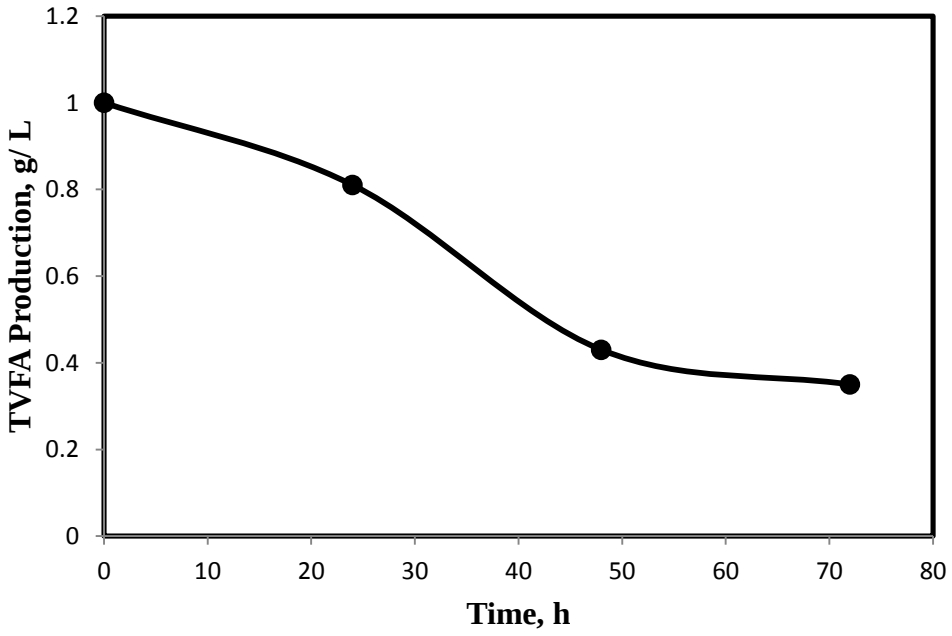


Figure 3.50 Variation of total volatile acids production (g/ L) with time at TSo=25 g/ L

3.1.2.5 Hydrogen Production at 35 g/ L Initial Total Sugar

The final initial total sugar concentration examined in the thesis was 35 g/ L at HRT=3 h. The maximum hydrogen volume observed at this initial total sugar concentration was only 470 mL/ day within 20 h of the operation. However, volume

decreased to around 338 mL/ day for the rest of operation (Figure 3.51). The time required to reach the steady state extended to 96 h, although it was between 20 h - 40 h for the initial substrate concentrations between 10 g/ L - 25 g/ L. Hydrogen percentage in the total gas was nearly 15 % at steady-state. Hydrogen production rate ranged between 0.34-0.24 L H_2/ L/ day (Figure 3.52) and yield of formation was nearly 26 mL/ g TS. Effluent total sugar concentration was varied between 17-24 g/ L with substrate removal of 37 % (Figure 3.53). Produced TVFA concentration was nearly constant at 0.6 g/ L for the time period between t=48 h to t=96 h (Figure 3.54). The performance values obtained at this initial substrate concentration is considerably lower than the ones observed at other substrate concentrations. In the light of this fact, it can be concluded that initial substrate concentration of nearly 35 g/ L causes substrate inhibition.

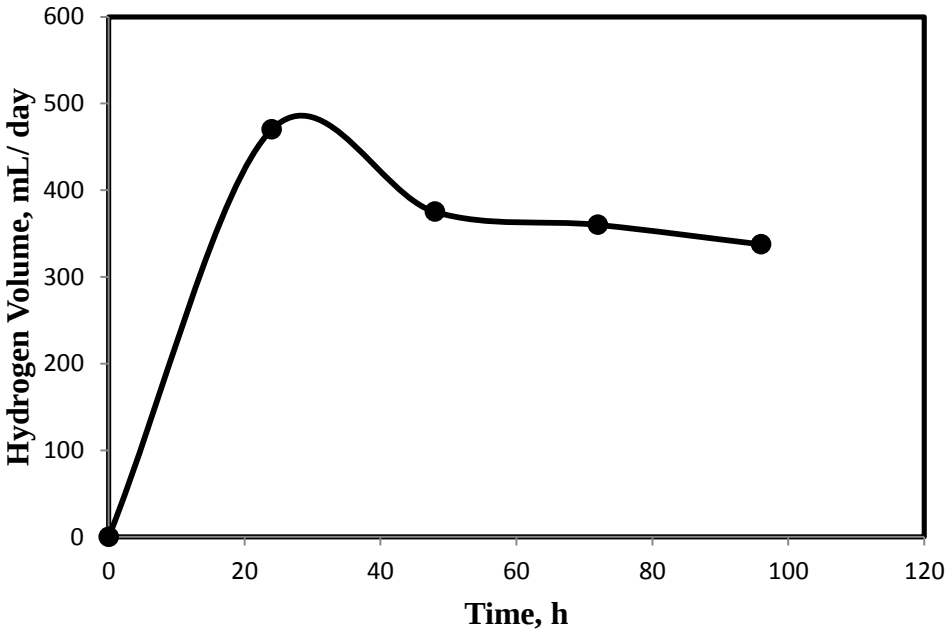


Figure 3.51 Variation of hydrogen gas volume with time at TSo=35 g/ L

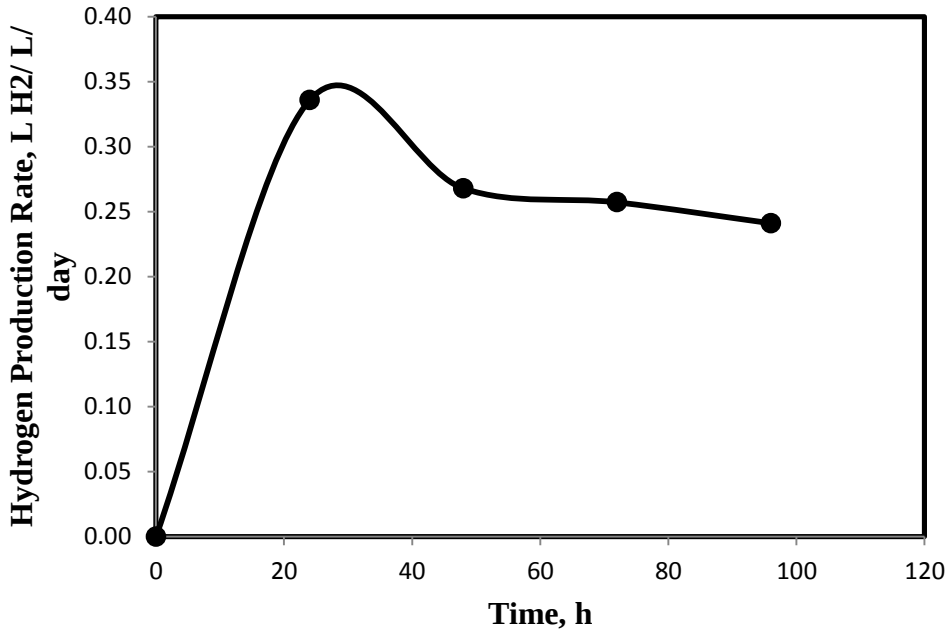


Figure 3.52 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with time at TSo=35 g/ L

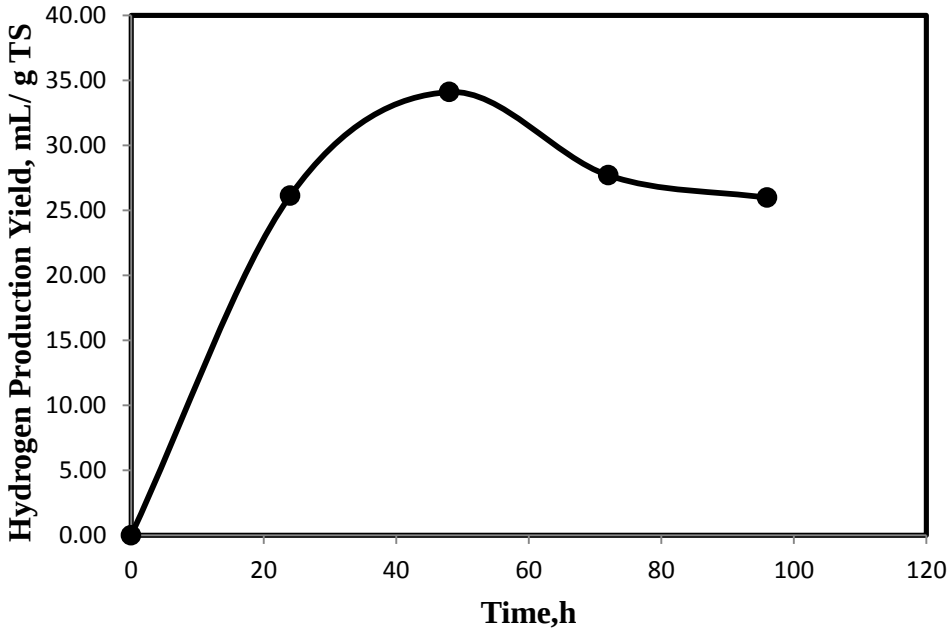


Figure 3.53 Variation of hydrogen production yield (mL/ g TS) with time at TSo=35 g/ L

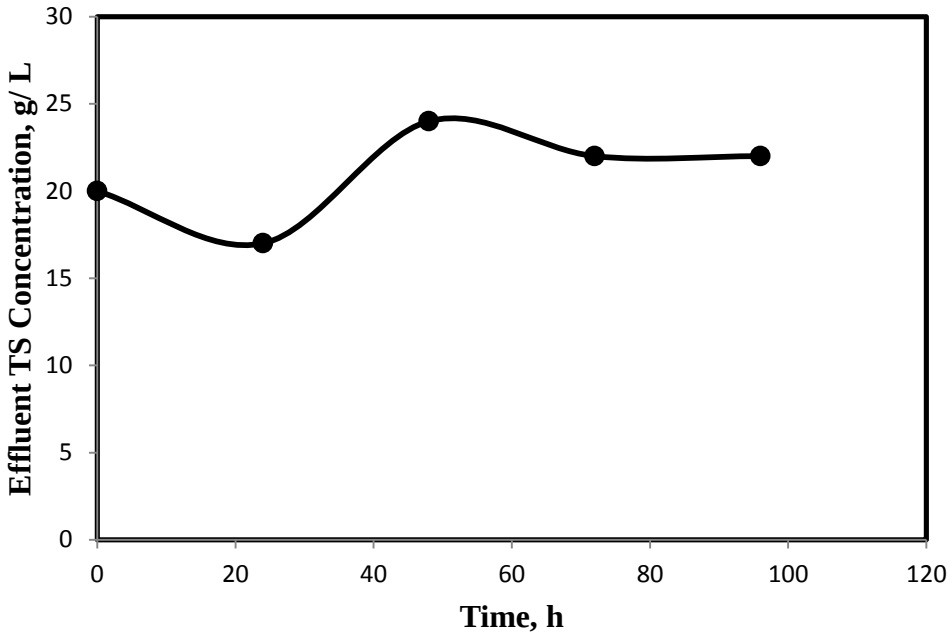


Figure 3.54 Variation of effluent total sugar concentration (g/ L) with time at TSo=35 g/ L

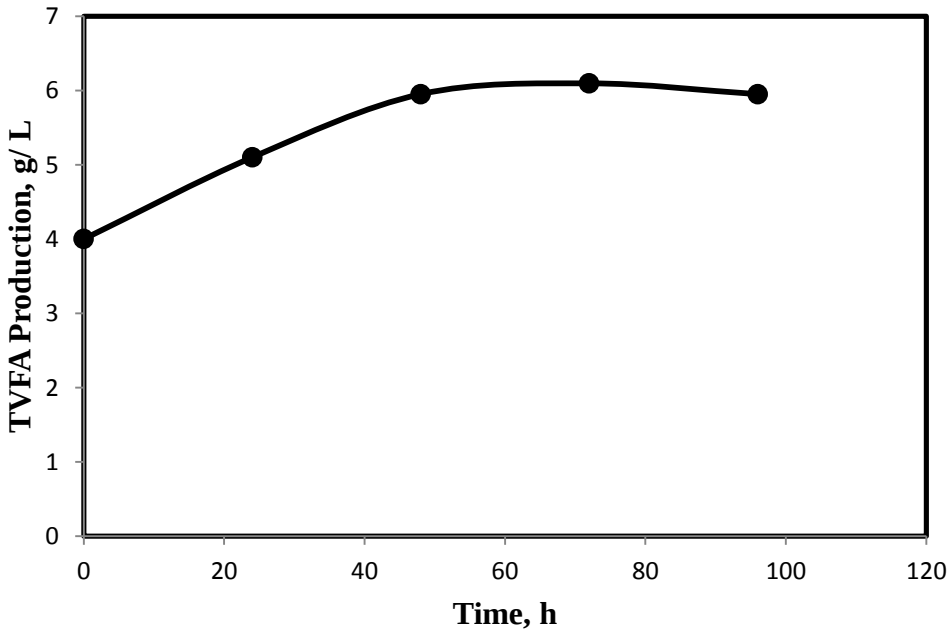


Figure 3.55 Variation of total volatile acids production (g/ L) with time at TSo=35 g/ L

3.2 The Overall Evaluation of Process

Pre-steady state conditions of hydrogen production at different hydraulic retention times and initial substrate concentrations were evaluated in the previous sections.

Variation of steady state hydrogen production performance of the system at different hydraulic retention times and initial substrate concentrations were summarized in this section. The effect of hydraulic retention time on daily hydrogen production volume at TSo= 15 g/ L is depicted in Figure 3.56. The volume was only 908 mL at HRT=1 h and increased to 2000 mL at HRT=2 h and then reached to maximum value of 3500 mL/ day at HRT=3 h (Figure 3.56). Higher HRTs resulted in decreasing in the production volume. Similarly, rate and yield of productions reached to maximum values at HRT=3 h. The yield was 645.69 mL/ g TS and the rate was 2.51 L H2/ L/ day (Figure 3.57 and 3.58, respectively). Although TVFA production was maximum at HRT=4.5, the hydrogen volume, rate and yield of productions were less than ones obtained at HRT=3 h (Figure 3.59). Therefore, HRT=3 h can be selected as the optimal retention time for the operated packed bed reactor.

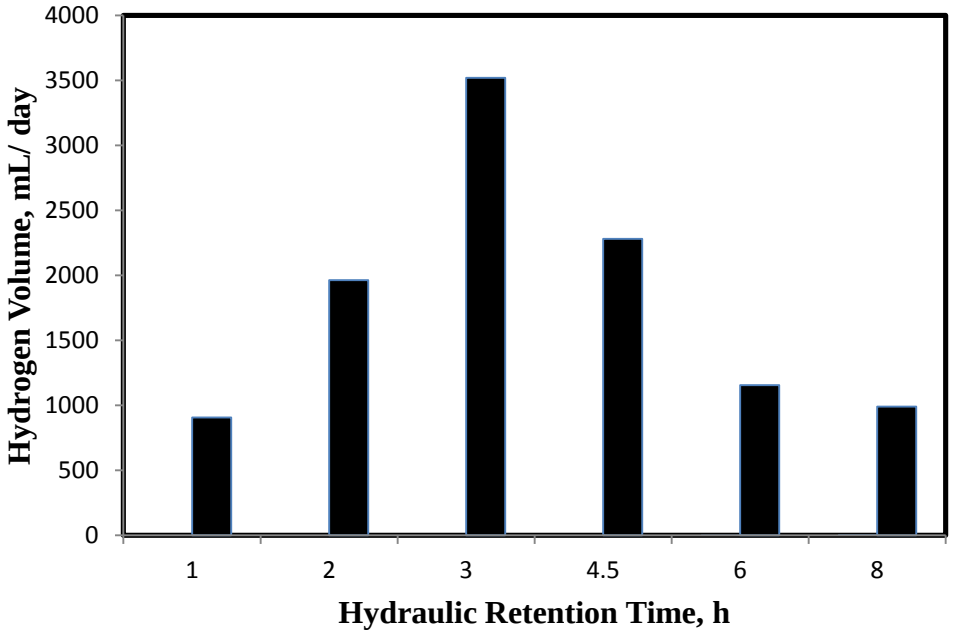


Figure 3.56 Variation of hydrogen gas volume with HRT

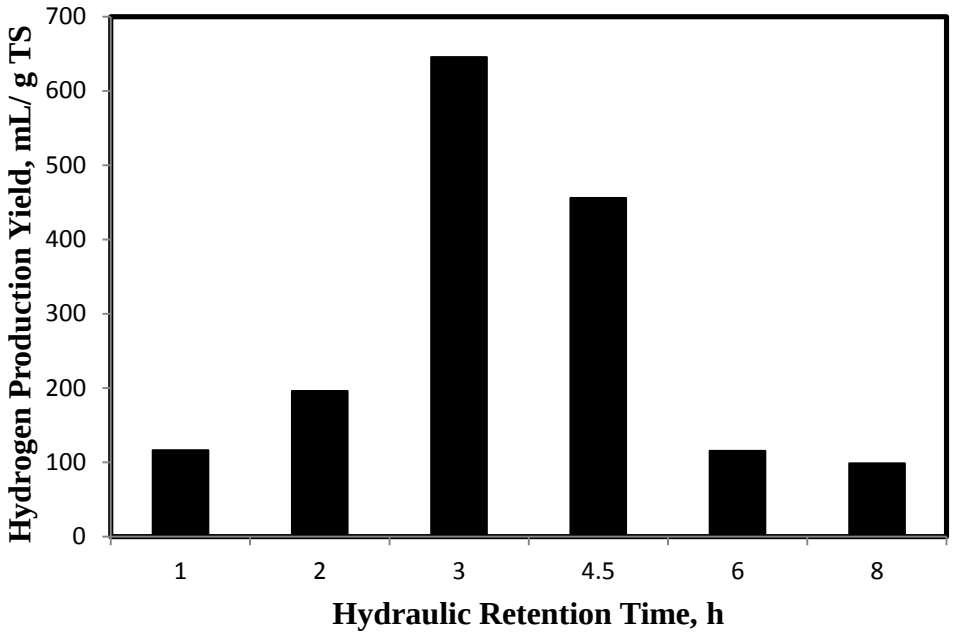


Figure 3.57 Variation of hydrogen production yield (mL/ g TS) with HRT

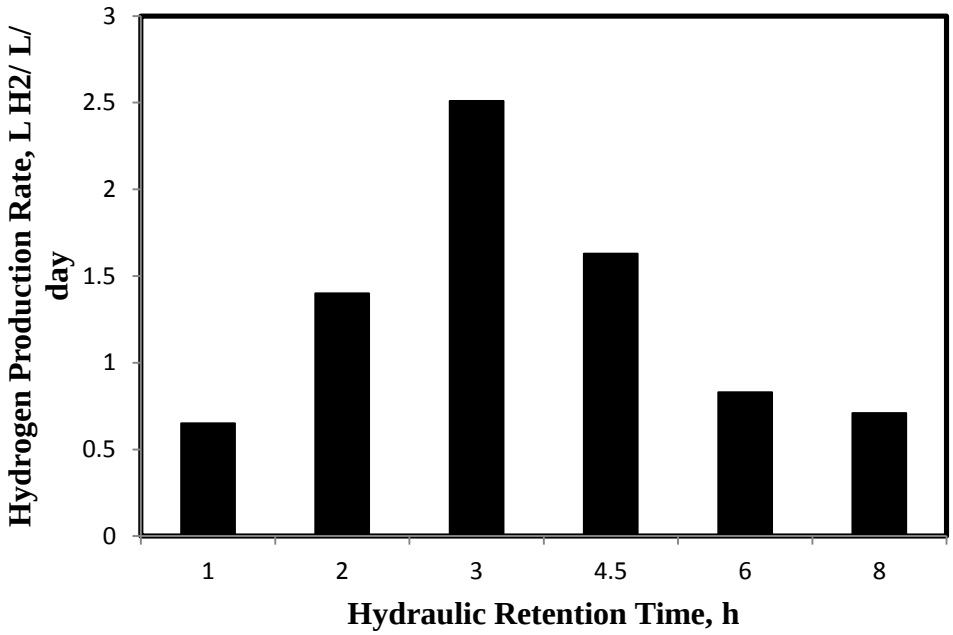


Figure 3.58 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with HRT

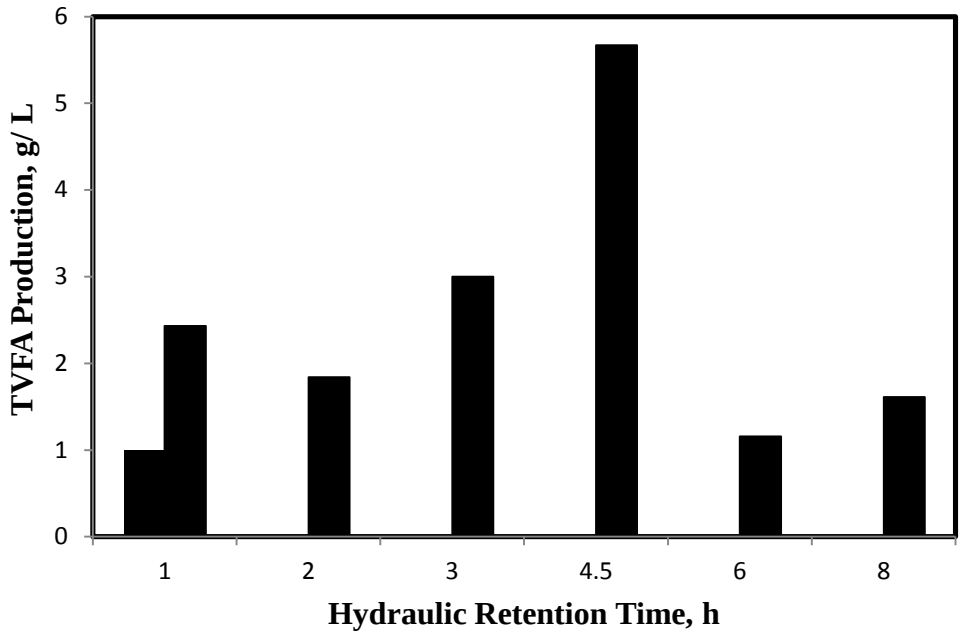


Figure 3.59 Variation of total volatile acids production (g/ L) with HRT

The effect of initial substrate concentration at HRT=3 h on the hydrogen production performance of the process were summarized through Figures 3.60 and Figures 3.64. The process performance of the process was very low at 10 g/ L initial substrate concentration. The volume and rate of production reached to maximum values as 4275 mL/ day and 3.05 L H_2/ L/ day respectively, when the system was operated at 25 g/ L total sugar concentration. However, production was substantially affected by substrate oncentration of TS_0=35 g/ L. In summary, low substrate concentration (10 g/ L) represents that the system was operated at substrate limited conditions. On the contrary, high substrate concentration of 35 g/ L causes substrate inhibition.

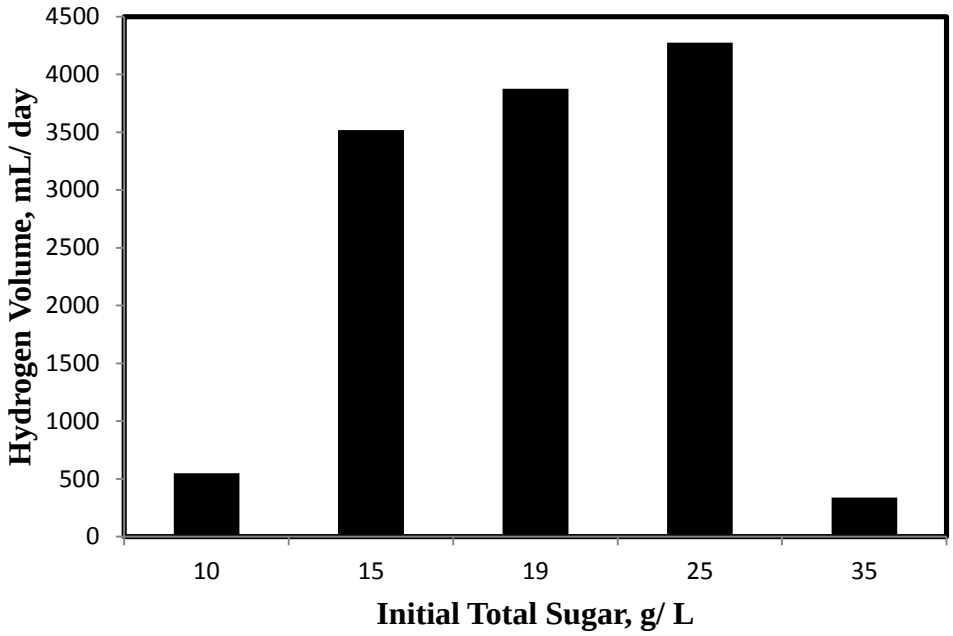


Figure 3.60 Variation of hydrogen gas volume with TSo

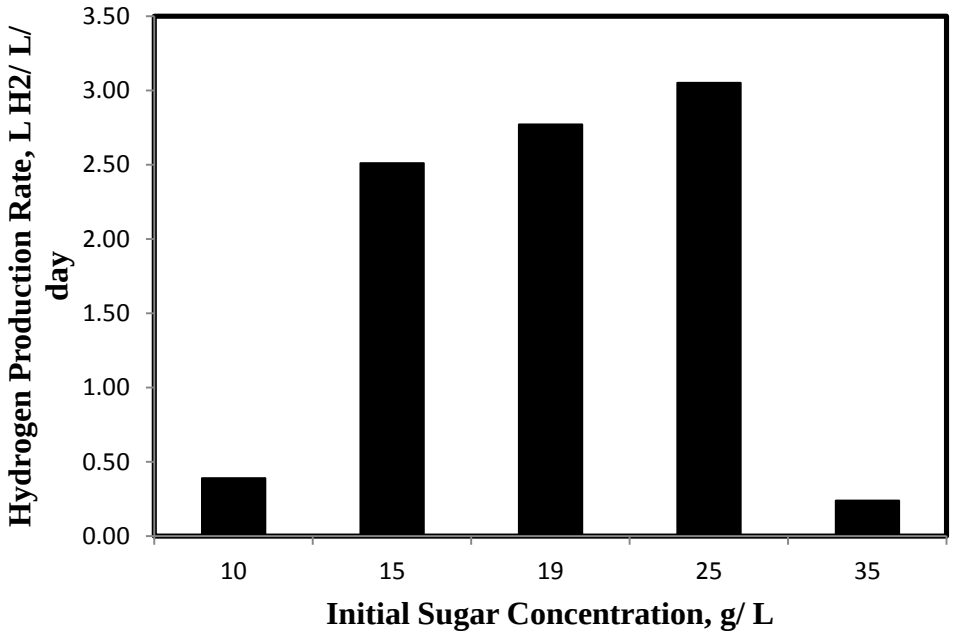


Figure 3.61 Variation of volumetric hydrogen production rate (L H_2/ L/ day) with TSo

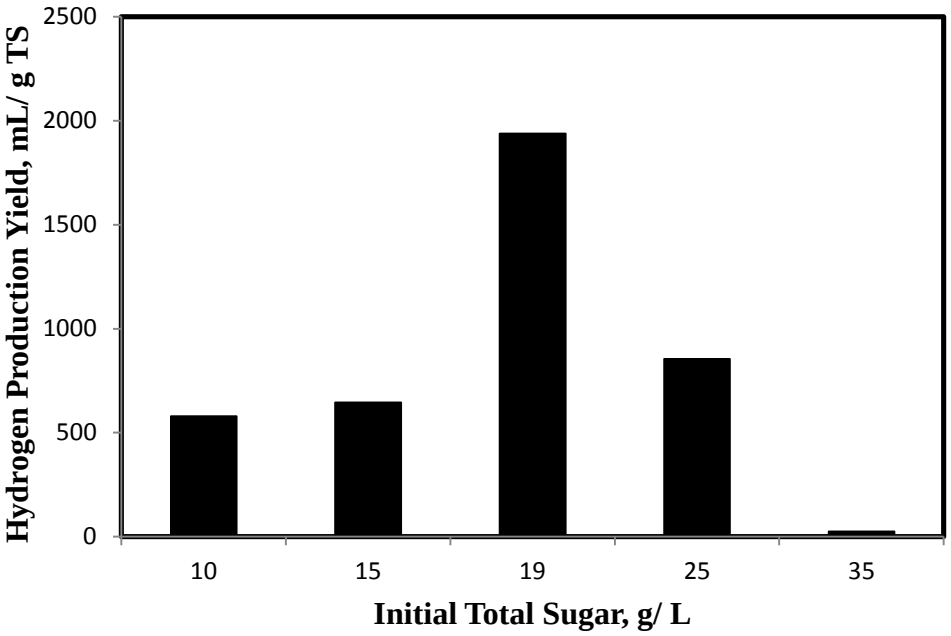


Figure 3.62 Variation of hydrogen production yield (mL/ g TS) with TSo

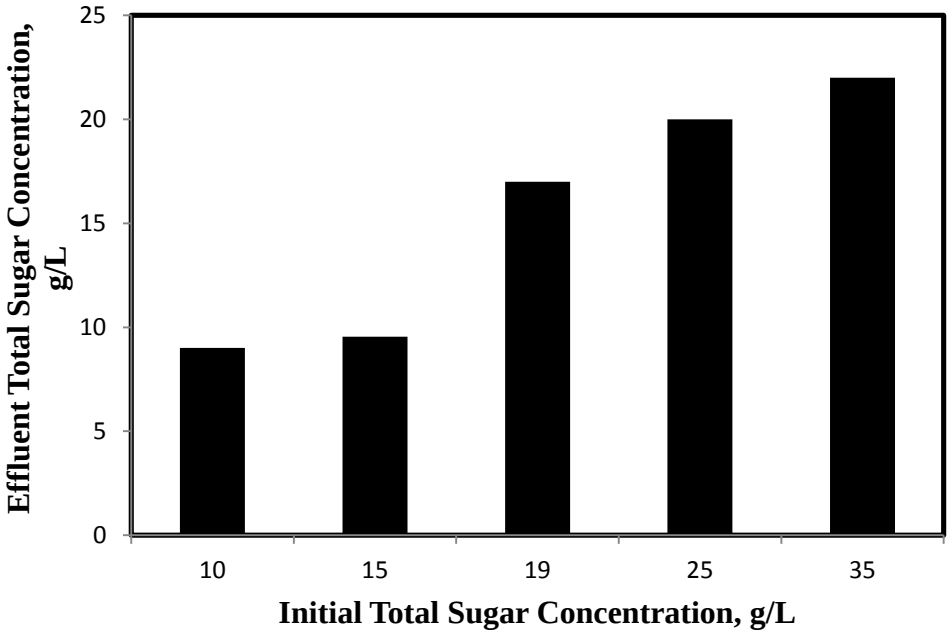


Figure 3.63 Variation of effluent total sugar concentration (g/ L) with TSo

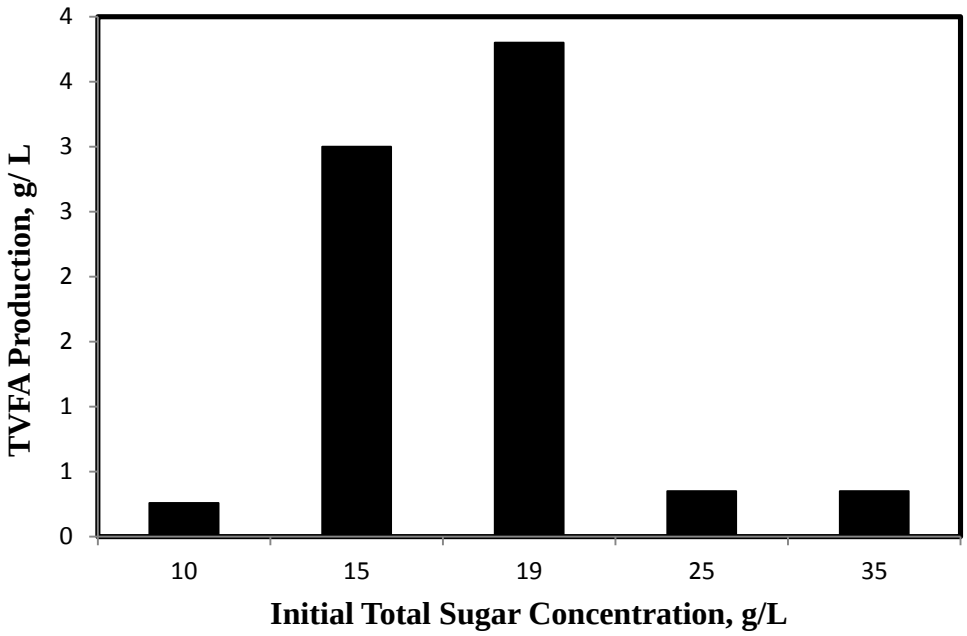


Figure 3.64 Variation of total volatile acids production (g/ L) with TSo

CHAPTER FOUR
CONCLUSIONS AND RECOMMENDATIONS

Hydrogen is a non-toxic, renewable, securely storable and transportable, economic and has got a high specific energy on a mass basis. The energy content of 9.5 kg of hydrogen is equivalent to that of 25 kg of gasoline. Apart from all these advantageous of hydrogen, it can be generated by different techniques and from various sources. The biological production of hydrogen, is considered as an important key sustainable world power supply and is currently being seen as the versatile fuel of the future, with the potential to replace fossil fuels.

The major research needs to advance biohydrogen production are development of process technology to increase the production yield and rate to be able to compete with other physical and chemical hydrogen production techniques, to use cost effective sugar rich substrates as starch or cellulose containing solid wastes and some food industry wastewaters, to determine pretreatment conditions of these wastes to convert their complex structures to the easily available form by microorganisms. Determining operating conditions specific to the substrate and reactor configuration are vital research subjects to advance the biohydrogen production technologies. It is well known that immobilized systems are superior over suspended growth bioprocess technologies. Support particle type used in immobilized system and optimization of operation conditions for efficient biohydrogen production are the main research subjects in immobilized growth systems.

In the light of aforementioned facts, biohydrogen production from hydrolyzed waste wheat powder by continuously operated dark fermentation process containing novel support material were investigated in this study. For this purpose, wheat powder was hydrolyzed by under acidic conditions (pH=2) and at high temperature (90 ^{0}C). The study was designed to determine optimum hydraulic retention time and initial substrate concentration for maximum hydrogen production yield and rate in up-flow packed bed reactor containing biological aquarium sponge support particles.

Hydrogen production at short hydraulic retention times is required mainly for process economy. Nevertheless, short hydraulic retention times or high feeding rate

provide other advantageous during biological process for hydrogen production by dark fermentation. The TVFA generation is one of the main factors that determine optimal HRT for maximum hydrogen production. The desired condition is high TVFA formation mainly composed of acetic and butyric acid. The conditions necessary for high TVFA formation are presence of acid forming microbial culture in the system, sufficient amount of substrate and well controlled environmental conditions as pH=5.0 - 6.0. However high concentration of TVFA could cause product inhibition which cease hydrogen generation. Therefore, if efficient TVFA production is achieved, it should be removed from the system continuously with high rate. Microorganisms should be protected against long term exposure to TVFA in order to overcome product inhibition and to control pH at 5.0-6.0. Short HRTs provide high substrate loading, short term exposure to TVFA and hence, control of pH at desired range. The liquid phase hydrogen is another inhibitory factor. After a certain concentration of hydrogen in the liquid phase, the metabolism of organisms can shift to the non-hydrogen generating end products as ethanol, hydrogen is consumed to produce propionic acid or homoacetogens become active to generate acetic acid from CO_2 and H_2. In order to overcome this problem, liquid phase hydrogen should be removed immediately. Short HRTs help the separation of hydrogen from the liquid phase to the head space. As flow-rate is increased, the linear liquid velocity in the process increases, then, hydrogen gas bubbles in the liquid phase are forced upwards to the head space. On the other hand, short HRTs or high dilution rates could cause wash out of organism form the process and high substrate loadings which could lead to substrate inhibition and cessation of hydrogen generation.

The support particle type is another significant factor to achieve efficient production in short HRTs. The support particles which retain high biomass, provide high void volume and long lasting ones are preferred. Therefore, the optimal HRT for the maximum production could vary depending on the support particle type.

The experimental results indicated that decreasing HRT from 8 hours to 3 hours at 15 g/ L initial total sugar concentration resulted in 32 times improvement in the yield of production, 3.5 times in the rate of production and 2 times more TVFA formation.

HRT=3 h provided the conditions as high TVFA formation but no long term exposure of organisms to TVFA and no TVFA inhibition, better separation of hydrogen and no hydrogen inhibition. Decreasing HRT to 1 h caused a substantial failure in all performance criteria due to wash out of organisms form the process. The other factor is the high rate of substrate loading which caused substrate inhibition. In other words, the system was operated at very high substrate loading rates, but there was no enough biomass to process the substrate. The organisms were washed out from the system with the effect of shear force during high linear velocity at low HRTs or high feeding rates.

Further improvement in the process was achieved by optimizing initial substrate concentration. The rate and yield of production at TS_0= 15 g/ L was 2.51 L H_2/ L/ day and 645.7 mL/ g TS. The percentage of hydrogen in the total gas volume was 50 ± 1 % and the daily hydrogen production was 3519 mL/ day. Increasing the initial substrate concentration to TS_0= 25 g/ L provided a substantial increase in the volume to 4275 mL/ day, hydrogen percentage in the total gas reached to the level of 82 % ± 1 %. The production rate was 3.05 L H_2/ L/ day. The maximum rate and production volume were obtained at 25 g/ L initial substrate concentration. Further increase in initial substrate caused substrate inhibition and hence resulted in decreasing in all performance criteria.

In summary, the results showed that using wheat powder as substrate for continuous, low-cost and high-yield of biohydrogen production in anaerobic packed bed reactor is feasible. Immobilized reactor system is suitable for high yield and rate of hydrogen generation. The aquarium biological sponge particles could be preferred as support particles due to its high porosity characteristic which may help both high biomass holding and gas transfers form liquid to head space. The optimal conditions to obtain maximum rate and yield of formation for dark fermentation of hydraulised wheat in aquarium biological sponge particles containing up-flow anaerobic packed bed reactor are HRT= 3 h and TSo= 25 g/ L.

Enhancing biohydrogen production from waste wheat by dark fermentation is a major challenge. Utilization of waste wheat in biohydrogen production will help waste minimization and sustainable energy production. As shown in this thesis,

immobilization is very advantageous than other growth systems in biohydrogen production. Although the yield and rate of production obtained under optimized conditions in this study is satisfactory, process could be further improved to increase hydrogen production volume, rate and yields. Some of the recommendations for that purpose are listed below:

- The effect of support particle number on biohydrogen production rate should be investigated.
- Lignocellulosic material can be used as substrate instead of waste wheat.
- The hydrogen production performance of pure microbial cultures from waste wheat by dark fermentation can be investigated in continuously operated paced bed reactor
- Effluent of dark fermentation process can be used as substrate in photo fermentation to increase the hydrogen production.

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APPENDICES

ABBREVIATIONS

ABS	Aquarium biological sponge
AC	Activated carbon
AFBR	Anaerobic fludizied bed reactor
AHWP	Acid hydrolysed wheat powder
ALSC	Acrylic latex plus silicone
ANS	Anaerobic sludge
CSTR	Continuous stirred tank reactor
DTFBR	Draft tube fludizied bed reactor
EC	Expanded clay
HRT	Hydraulic retention time
LS	Loofah sponge
ORP	Oxidation reduction potential
TSo	Initial total sugar
TS	Total sugar
TVFA	Total volatile fatty acids
UASB	Up-flow anaerobic sludge blanket bioreactor
UPBR	Up-flow packed bed reactor

A.1 Raw data for the Variable Hydraulic Retention Time (HRT) Experiments

Table A.1.1 Raw data for HRT = 1 hours

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	1400	15000	12000	3000	4.4	-210	36	0	53	53000	0	0.00	0.00	5280	7940	2660
24	1400	16000	7200	8800	4.8	-227	35	5950	33	33000	1964	223.13	1.40	5840	8060	2220
48	1400	16000	8200	7800	4.6	-227	34	6050	15	15000	908	116.35	0.65	5800	8180	2380
72	1400	16000	8200	7800	4.5	-158	35	6050	15	15000	908	116.35	0.65	5770	8200	2430

Table A.1.2 Raw data for HRT = 2 hours

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	700	15000	4500	10500	4.4	-75	37	0	25	25000	0	0.00	0.00	4400	6180	1780
24	700	15000	4300	10700	5.0	-94	35	5300	27	27000	1431	133.74	1.02	4500	6980	2480
48	700	15000	5000	10000	5.3	-178	34	5300	37	37000	1961	196.10	1.40	4280	7500	3220
72	700	15000	5000	10000	5.2	-205	34	5300	37	37000	1961	196.10	1.40	5660	7500	1840

Table A.1.3 Raw data for HRT = 3 hours

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	467	15000	8800	6200	5.8	-130	35	0	42	42000	0	0.00	0.00	3600	6180	2580
24	467	15000	8850	6150	5.2	-170	36	6300	48	48000	3024	491.71	2.16	3830	6330	2500
48	467	15000	9200	5800	4.8	-150	38	6900	50	50000	3450	594.83	2.46	3540	6680	3140
72	467	15000	9550	5450	4.5	-190	36	6900	51	51000	3519	645.69	2.51	3440	6440	3000

Table A.1.4 Raw data for HRT = 4.5 hours

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	310	15000	4200	10800	5.1	-180	35	0	12	12000	0	0.00	0.00	1980	3300	1320
24	310	16000	7400	8600	5.4	-280	37	1700	26	26000	442	51.40	0.32	2000	5110	3110
48	310	15000	7100	7900	4.7	-176	38	1800	13	13000	234	29.62	0.17	2000	8740	6740
72	310	15500	9600	5900	5.2	-215	36	3550	42	42000	1491	252.71	1.07	2000	7630	5630
96	310	15500	10000	5500	5.3	-270	35	3700	63	63000	2331	423.82	1.67	2150	7750	5600
120	310	15000	9800	5200	5.3	-220	35	3550	65	65000	2307.5	443.75	1.65	2100	7630	5530
144	310	15000	9500	5500	5.1	-180	36	3750	61	61000	2287.5	415.91	1.63	2190	7500	5310
168	310	15000	10000	5000	4.9	-150	36	3800	60	60000	2280	456.00	1.63	2140	7810	5670

Table A.1.5 Raw data for HRT = 6 hours

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	230	15000	6000	9000	4.8	-250	36	1050	53	53000	0	0.00	0.00	360	1050	690
24	230	15000	5000	10000	5.1	-250	36	1150	62	62000	713	71.30	0.51	374	1094	720
48	230	15000	5500	9500	5.1	-130	35	1600	61	61000	976	102.74	0.70	472	1220	748
72	230	15000	5000	10000	4.8	-110	36	1700	66	66000	1122	112.20	0.80	514	1552	1038
96	230	15000	5000	10000	4.9	-160	37	1750	65	65000	1137.5	113.75	0.81	622	1636	1014
120	230	15000	5000	10000	5.4	-235	37	1750	66	66000	1155	115.50	0.83	964	2120	1156

Table A.1.6 Raw data for HRT = 8 hours

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	175	14000	10000	4000	4.8	-50	37	0	8	8000	0	0.00	0.00	6600	8240	1640
24	175	14000	8000	6000	5.6	-300	30	1300	11	11000	143	23.83	0.10	6600	8660	2060
48	175	15000	5000	10000	4.9	-200	34	2800	27	27000	756	75.60	0.54	6360	8340	1980
72	175	15000	6000	9000	5.5	-193	34	3000	33	33000	990	110.00	0.71	6360	8220	1860
96	175	14000	4000	10000	5.0	-210	37	3200	31	31000	992	99.20	0.71	7520	9260	1740
120	175	14000	4000	10000	5.3	-200	38	3000	33	33000	990	99.00	0.71	7520	9130	1610

A.2 Raw data for the Variable Initial Total Sugar (TSo) Experiments

Table A2.1 Raw data for TSo = 10 g/ L at HRT = 3 h.

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	467	10000	5800	4200	4.6	-160	38	0	55	55000	0	0.00	0.00	950	1130	180
24	467	10000	7000	3000	5.2	-135	37	2500	22	22000	550	183.33	0.39	900	1260	360
48	467	10000	9160	840	5.1	-170	37	2500	23	23000	575	684.52	0.41	870	1096	226
72	467	10000	9050	950	5.1	-236	36	2500	22	22000	550	578.95	0.39	890	1150	260

Table A2.2 Raw data for TSo = 15 g/ L at HRT = 3 h.

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	467	15000	8800	6200	5.8	-130	35	0	42	42000	0	0.00	0.00	3600	6180	2580
24	467	15000	8850	6150	5.2	-170	36	6300	48	48000	3024	491.71	2.16	3830	6330	2500
48	467	15000	9200	5800	4.8	-150	38	6900	50	50000	3450	594.83	2.46	3540	6680	3140
72	467	15000	9550	5450	4.5	-290	36	6900	51	51000	3519	645.69	2.51	3440	6440	3000

Table A2.3 Raw data for TSo = 19 g/ L at HRT = 3 h.

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	467	19000	14200	4800	4.9	-150	39	0	60	60000	0	0	0.00	1960	6180	4220
24	467	19000	6600	12400	5.2	-280	39	4050	63	63000	2551.5	206	1.82	3830	6330	2500
48	467	19000	10000	9000	5.0	-216	38	7550	51	51000	3850.5	428	2.75	3320	6440	3120
72	467	19000	17000	2000	5.3	-205	38	7600	51	51000	3876	1938	2.77	2410	6220	3810
96	467	19000	17000	2000	5.1	-195	39	7750	50	50000	3875	1938	2.77	2400	6200	3800

Table A2.4 Raw data for TSo = 25 g/ L at HRT = 3 h.

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	467	25000	20000	5000	4.8	-85	35	0	35	35000	0	0.00	0.00	1000	2000	1000
24	467	25000	20000	5000	4.9	-180	36	3150	36	36000	1134	226.80	0.81	1100	1910	810
48	467	25000	19000	6000	5.3	-200	36	5150	82	82000	4223	703.83	3.02	950	1380	430
72	467	25000	20000	5000	4.9	-162	36	5150	83	83000	4274.5	854.90	3.05	1020	1370	350

Table A2.5 Raw data for TSo = 35 g/ L at HRT = 3 h.

Hour	Q (mL/ h)	TSo (mg/ L)	TSe (mg/ L)	Total Sugar Consumption (mg/ L)	pH	ORP	T (°C)	Total Gas Volume (mL)	% Hydrogen Gas	Hydrogen gas concentration (ppm)	Total Hydrogen Gas Volume (mL)	Hydrogen Production Yield (mL/ g TS)	Hydrogen Production Rate (L H_2/ L/ day)	TVFAo (mg/ L)	TVFAe (mg/ L)	Produced TVFA
0	467	35000	20000	15000	5.1	-236	36	0	22	22000	0	0.00	0.00	1000	5000	4000
24	467	35000	17000	18000	5.1	-165	36	1000	47	47000	470	26.11	0.34	810	5910	5100
48	467	35000	24000	11000	5.2	-206	35	1250	30	30000	375	34.09	0.27	430	6380	5950
72	467	35000	22000	13000	5.0	-145	36	2250	16	16000	360	27.69	0.26	325	6420	6095
96	467	35000	22000	13000	5.3	-183	36	2250	15	15000	337.5	25.96	0.24	380	6330	5950