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DEVELOPMENT OF BIO-REACTOR FOR THE PRODUCTION OF HYDROGEN FROM PLANT BIOMASS

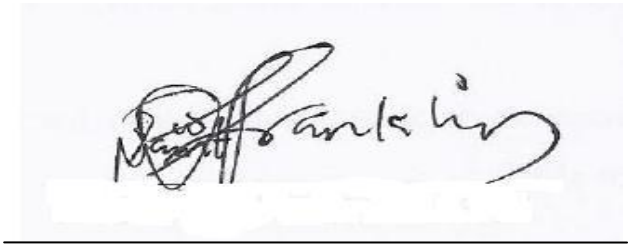
FRANKLIN OCHUKO OBAZU

A THESIS SUBMITTED TO THE FACULTY OF SCIENCE, UNIVERSITY OF THE WITWATERSRAND, IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN SCIENCE.

JOHANNESBURG, 2012.

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



Franklin Ochuko OBAZU

25th day of October, 2012

ABSTRACT

The research objectives for this study involved the development of a modified thermophilic fluidized bacterial granular bed bioreactor system for the production of biohydrogen from sucrose. The granules were comprised of an undefined anaerobic thermophilic multispecies consortium of bacteria. In order to establish the thermophilic bacterial granules, the bioreactor was operated as a chemostat under increasing dilution rates. This promoted the selection and enrichment of thermophilic granules comprised of a multispecies bacterial consortium. Endo medium which is one of the most basic bacteriological nutrient mediums was used as the nutrient supply in the granule generating chemostat experiments. Bacterial inoculums from mesophilic environments were used to induce and establish thermophilic and extreme-thermophilic adapted bacterial granules in the chemostat experiments. Granulation was successfully induced under a thermophilic temperatures ranging from 55 ^{o}C to 70 ^{o}C within a period ranging from 5 to 14 days. Bioreactor design and operation was modified so as to increase both hydrogen yield (*HY*) and volumetric hydrogen productivity (*HP*). It was found that in order to increase both *HY* and *HP* it was necessary to implement a number of modifications in bioreactor design and operation. The two key operational parameters were temperature and de-gassed effluent recycling rate through the bioreactor bed. Through the incorporation of a solid-liquid separator in the form of 11.6 L settling column, bacteria granular bed wash out was prevented for a 5.0 L thermophilic bioreactor system operated at high volumetric biomass densities, low hydraulic retention times and high degassed effluent recycle rates. Stability of the bioreactor operation in terms of volumetric hydrogen productivity (L H_2/L/h), %H_2 content and pH maintenance was

readily maintained for 50 days. While volumetric hydrogen productivity increased with bacterial biomass density, both hydrogen yield (mol H_2/mol glucose) and specific hydrogen productivity (L H_2/g/h) declined with increasing biomass density. In this process the rate of physical removal of H_2 trapped in the bulk liquid phase surrounding the fluidized granules reduced the thermodynamic constraints preventing the simultaneous achievement of high *HPs* and high *HYs* in a granular fluidized bed derived from an undefined bacterial culture.

It became evident that a thermophilic temperature alone was an insufficient condition to achieve simultaneously high *HPs* and high *HYs*. It also became evident that hydraulic retention time for degassed effluent recycling was a critical for the simultaneous achievement of high *HPs* and high *HY*. It was discovered that a reduction in the total volume of bioreactor system relative to increasing rates of degassed effluent recycle was a necessary condition for the simultaneous achievement of both high *HPs* and high *HYs*. Thus at thermophilic temperatures any increase in the bioreactor system volume should also be accompanied by a concomitant increase in the rate of degassed effluent recycling so the *HRT* always remained below the critical threshold necessary for the simultaneous achievement of high *HPs* and high *HYs*.

Once it was demonstrated that by the adjusting bioreactor system volume and the degassed to effluent recycle rates both high *HPs* and high *HYs* could be achieved only under thermophilic conditions it was necessary to show that under these operational condition the system would produce net positive work in terms of hydrogen energy production. It was shown through modeling heat exchanges that if the bioreactor was

effectively insulated and waste heat was recycled or recovered then net positive work was accomplished by the bioreactor system.

Bacterial granules grown from mesophilic inoculant were adapted to generate H_2 from sucrose under a range of thermophilic temperatures (55, 60, 65, 70 ^{o}C). Attainments of two H_2 generation process goals were assessed. First, whether a net positive net energy balance at thermophilic temperatures and high effluent recycle rates were attainable. Secondly, whether the volumetric hydrogen productivities were sufficient to drive a 5 kW fuel cell when scale-up to 1 m^3

DEDICATION

This thesis is exclusively dedicated to our Lord Jesus Christ who is, who was and who is to come in the glory of God the Father, forever and ever. Amen.

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I ascribe all praise, glory and thanks to the Almighty God, the wonder, miracle and self-sufficient God who gave all enablement against all odds for making this study a befitting success. His grace is truly sufficient.

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Finally, I should end by saying that the order in which I have presented these acknowledgements has nothing to do with hierarchy, I owe many people appreciation, all of whom might not be acknowledged here, but I am in no doubt there will be opportunities outside the printed page to assure them of my deepest appreciation for their support.

RESEARCH OUTPUT

(A) ORIGINAL PUBLICATIONS

1. L. Ngoma, P. Masilela, **F. Obazu,** V.M. Gray 2011., The effect of temperature and effluent recycle rate on hydrogen production by undefined bacterial granules. Bioresource Technol. 102, 8986–8991 (Published)

2. **F.O. Obazu,** L. Ngoma, V. M. Gray 2012., Interrelationships between bioreactor volume, effluent recycle rate, temperature, pH, $\%H_2$, hydrogen productivity and hydrogen yield with undefined bacterial cultures. Int J Hydrogen Energy. 37 (7):5579–5590 (Published)

3. **Franklin O. Obazu,** Lubanza Ngoma, Vincent M. Gray, The influence of bacterial biomass density on hydrogen production efficiency in a thermophilic granular bed bioreactor. Biomass and Bioenergy J (Submitted)

4. Lubanza Ngoma, **Franklin Ochuko Obazu,** Vincent Myles Gray, Simultaneous achievement of high hydrogen yields and high volumetric productivities for biohydrogen production. Int J Hydrogen Energy (Submitted)

5**. F.O. Obazu,** L. Ngoma, V. M. Gray, Biohydrogen production by bacterial granules adapted to grow at different thermophilic temperatures. Int J Hydrogen Energy (Submitted)

(B) PATENTS

2010/04063- Provisional- 54: Effluent Gas-Disengager-71:1. University Of The Witwatersrand Johannesburg-72:1.Dr Vincent Myles Gary 2. Dr Lubanza Ngoma 3. Phumlani Masilela 4. **Franklin Ochuko Obazu**-33: United States of America- 31: 61/010,447-32: 08101/2008.

(C) CONFERENCE OUTPUT

1. **F.O. Obazu** and V.M. Gray The development of bioreactor for the production of hydrogen from plant biomass. The 3rd Cross Faculty Symposium, 26 – 29th October 2010 Wits University, Johannesburg (Poster Presentation)

2. **F.O. Obazu** and V.M. Gray Hyvolution Conference Presentation on Dark Fermentation in Amsterdam, Netherlands from the 15 – 19th of December 2010 (Oral Presentation)

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LIST OF ABBREVIATIONS

a	Specific interfacial area per unit volume of liquid in the bioreactor
AFGB	Anaerobic fluidized granular bed bioreactor
B	Working volume of bioreactor
B_{H_2}	Hydrogen power (W) output of AFGB system
CAC	Cylindrical activated carbon particles
C_p	Molar specific heat(J/K/mol) at constant pressure for a working fluid mixture
C_{pCO_2}	Specific heat capacity for CO_2
C_{pH_2}	Specific heat capacity for H_2 gas
C_{pH2O}	Specific heat capacity for water vapour
C_{pw}	Specific heat of water
C_v	Molar specific heat (J/K/mol) at constant volume for a working fluid mixture
D	Dilution rate
e_s	Equilibrium saturated vapour pressure within effluent gas disengager
E	Rate of water vapour loss from the effluent gas disengager
λE	Latent heat loses from the effluent gas disengager
F_{er}	Degassed effluent recycle rate
F_{ir}	Influent or nutrient supply flow rate
g	Gravitational acceleration constant
G	Volume of effluent gas disengage
G_{H_2}	Total H_2 gas flow rate from the effluent gas-disengager
G_T	Total gas production rate from the gas meter measurements
h	Hours

H	Sensible or convective heat loses from the AFGB system
$\%H_2^{GC}$	Percentage hydrogen content from GC measurements
H_2^L	Super-saturated total concentration of H_2 in the liquid phase
H_2^{L*}	Thermodynamic equilibrium concentration of H_2 dissolved in the liquid phase
$(H_2^L)_G$	Represents the concentration of super-saturated H_2 transferred into gas phase
$(H_2^L)_L$	Represents the concentration of super-saturated H_2 trapped in the liquid phase
$(H_2^{L*})_L$	Thermodynamic equilibrium concentration or H_2 entering the bioreactor via the recycled effluent stream
H_b	Actual height to which the fluid is pumped within the bioreactor system
H_{er}	Head rise for degassed effluent recycling pump
H_{ir}	Head rise for the nutrient supply or influent pump
HP	Volumetric hydrogen productivity
HRT	Hydraulic retention time
HY	Hydrogen yield
F_{er}	Degassed effluent recycle rate
k_L	Liquid phase volumetric mass transfer coefficient or film coefficient
$k_L a$	Liquid phase volumetric mass transfer coefficient for H_2 in aqueous solutions
K_H^T	Henry's constant for dissolved H_2
$\dot{m}_{CO_2}$	CO_2 mass flux rate from the effluent gas disengager
$\dot{m}_{H_2}$	H_2 mass flux rate from the effluent gas disengager
$\dot{m}_{H20}$	Water vapour mass flux rate from the effluent gas disengager
P	Total volume of piping for the AFGB system
P_a	Ambient atmospheric pressure (kPa) for Johannesburg, South Africa

P_{dp}^{er}	Discharge pressure of the degassed effluent recycle pump
P_{dp}^{ir}	Discharge pressure of the nutrient supply or effluent pump
P_{er}	Electrical power required for effluent recycling
P_{in}	Initial pressure of the mixed gas flux existing the effluent gas disengager
P_{ir}	Electrical power required for influent supply
P_H	Partial pressure of hydrogen
P_{ir}	Electrical power required for pumping nutrients into bioreactor
Q_{CO_2}	Waste heat energy loss associated with CO_2 fluxes from the gas disengager
Q_{so}	Heat energy recovered from the effluent overflow
Q_{H_2}	Waste heat energy loss associated with H_2 fluxes from the gas disengager
Q_{H2O}	Waste heat energy loss associated with vapour fluxes from the gas disengager
Q_{hp}	Heat energy delivered from the heat-pump
Q_i	Electrical power required for heating nutrient influent
Q_{waste}	Waste heat released from the effluent gas disengager
R	Universal gas constant
R_e	Radiant energy emission from AFGB system
S	Separator volume of AFGB system
SGC	Specific glucose consumption rate
SHP	Specific hydrogen productivity per g of bacterial biomass
STY	Space/time yields per unit bioreactor volume
T_a	Ambient temperature
T_b	Bioreactor temperature
T_{in}	Initial temperature of the mixed gas flux exiting the effluent gas disengage
T_{out}	Temperature of mixed gas flux exiting the heat-pump
V	Total fluid filled volume of the AFGB system

V_{er}	Linear degassed effluent flow velocity
V_{ir}	Linear influent flow velocity
V_G	Represents the total gaseous phase volume of the bioreactor
W_{hp}	Electrical power required for the operation of the heat-pump
W_{net}	Net work produced by the bioreactor system
η_{hp}	Heat pump energy transformation efficiency, electrical energy into heat energy
η_m	Dimensionless electrical motor efficiency
η_p	Dimensionless pumping efficiency
ρ_B	Bacterial granular biomass density
λ	Latent heat of evaporation constant
ρ_{EH_2}	Volumetric hydrogen energy density
ρ_{H_2}	Density of the H_2 in the gaseous phase
ρ_w	Density of water

CHAPTER ONE

Introduction

1.1 Background and Motivations

Fossil fuels are not renewable and will be exhausted sooner than we imagine. In addition, the use of fossil fuels has induced very severe environmental pollution. Hence, it is necessary to find alternative energy sources that are renewable and environmentally friendly (Das *et al*., 2008, 2001). Energy is vital to global prosperity, yet dependence on fossil fuels as our primary energy source contributes to global climate change, environmental degradation and health problems (Bockris, 2002). Pertinently, mankind is facing significant energy challenges, hence biohydrogen (Angelidaki *et al*., 2007). Hydrogen is the most abundant element in the universe and it is a clean, recyclable, and efficient new energy carrier (Das *et al*., 2001; Sushmita Mohapatra , 2012 Masset *et al*., 2012;). Hydrogen can be produced through various ways, which makes it renewable. When hydrogen is combusted as a fuel or converted to electricity, it produces water only consequently making it environmentally friendly (Hawkes *et al*., 2002 and Kapdan *et al*., 2006; Karthic Pandu and Shiny Joseph, 2012). Among various hydrogen producing processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure (Kraemer *et al*., 2006 and Nishio *et al*., 2004).

Biological methods for production of hydrogen mainly include: direct and indirect biophotolysis, fermentation, photosynthetic production and also in vitro enzymatic conversion of biomass are significant (Woodward *et al.*, 1996). Table 1.1 summarizes the

relative advantages and disadvantages of some important biological hydrogen production processes.

Although, hydrogen can easily be produced by water electrolysis, thermo chemical, radiolytic and biological processes, they are energy-intensive and economically viable only in areas where electricity is cheap (Rajeshwar *et al*., 1994). However, hydrogen has the highest gravimetric energy density of any known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing carbon-based emissions that contribute to environmental pollution and climate change.

Table 1.1: Comparison of important biological hydrogen production processes (Nath and Das, 2004)

Process	**Type of microorganisms**	**Advantages**	**Disadvantages**
Direct biophotolysis	Green algae	Can produce H_2 directly from water and Sunlight Solar conversion energy increased by tenfolds as compared to trees, crops	Requires high intensity of light O_2 can be dangerous for the system
Indirect biophotolysis	Cyanobacteria	Can produce H_2 from water Has the ability to fix N_2 from atmosphere	Lower Photochemical Efficiency Uptake hydrogenase enzymes are to be removed to stop degradation of H_2 About 30% O_2 present in gas mixture O_2 has an inhibitory effect on nitrogenase
Photofermentation	Photosynthetic bacteria	A wide spectral light energy can be used by these bacteria Can use different waste materials like distillery effluents, waste etc	Light conversion efficiency is very low, only 1–5% O_2 is a strong inhibitor of hydrogenase
Dark fermentation	Fermentative bacteria	It can produce H_2 all day long without light A variety of carbon sources can be used as Substrates It produces valuable metabolites such as butyric, lactic and acetic acids as by products It is anaerobic process, so there is no O_2 limitation problem	Relatively lower achievable yields of H_2 As yields increase H_2 fermentation becomes thermodynamically unfavorable Product gas mixture contains CO_2 which has to be separated

Hydrogen fuel cells and related hydrogen technologies provide the essential link between renewable energy sources and sustainable energy services. The transition from a fossil fuel-based economy to a hydrogen energy-based economy, however, is a fraught with many technical challenges, from the production of sufficient quantities of hydrogen to its storage, transmission, and distribution (Dunn, 2002).

1.1.1 Comparison of H_2 and Other Fuel

Hydrogen is considered to be an ideal source of energy for the future because it is easily converted to electricity by fuel cells, does not evolve the green house gas carbon-dioxide in combustion and is cleanly combustible. Among the many process of hydrogen production, microbial hydrogen synthesis is gaining momentum because it is an energy saving process (Nandi and Sengupta, 1998; Hallenbeck, 2011). However, irrespective of the merits of other fuel their demerits counter completely their essence and practice (table1.2). For instance the merits of the biodiesel given in the literature include domestic origin, reducing the dependency on imported petroleum, biodegradability, high flash point and inherent lubricity in the neat form (Mittelbach and Remschmidt, 2004; Knothe *et al*., 2005). But it's higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxides (NOx) emissions affects deeply it's practice (Demirbas, 2007).

Table 1.2: Comparison (merits and demerits) of H_2 and other fuel

Fuel	Merits	Demerits	Reference
Biodiesel	Very portable, ready availability, renewability, higher combustion efficiency, lower sulfur and aromatic content	higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxides (NOx) emissions, lower engine speed and power, injector coking, engine compatibility, high price and higher engine wear.	Ma and Hanna, 1999 Knothe *et al*., 2006 Demirbas, 2007
Bioethanol	It could reduce CO_2 emissions by 60-90 % relative to conventional petroleum fuels. CO_2 released during the combustion of bioethanol is recycled through the photosynthetic process resulting in no net increase to CO_2 levels	The combustion of neat ethanol (E100), contributes to the emission of aldehydes particularly acetaldehyde, which is between 2 to 4 times lower in gasoline emissions.	Brown *et al*., 1998
Biomethane	It's a well established process, the end-product of methanogenesis is a useful energy source; it is a low value end product with relatively less energy content (about 56 kJ energy/g CH_4).	methane and its combustion by-product are powerful greenhouse gases, and responsible for global climate change.	Shihwu, 2004
Biohydrogen	Hydrogen gas (H_2) is a clean fuel that possesses a high energy content per unit weight (122 KJ g-1) and does not contribute particulate or greenhouse gas emissions into the atmosphere upon combustion. No O_2 limitation problem and It produces valuable metabolites such as butyric, lactic, and acetic acids as by products	Still needs a practical process to extract nearly all of the hydrogen from the substrate (glucose) to yield $12H_2$/glucose, hence commercialization Still not feasible	Mizuno, O. *et al.*, 2000 Sinha, and Pandey, 2011 Nath and Das, 2004

Hydrogen seem to be the future focus, even when methane generation is usually one of the main end-products of wastewater treatment, the coupling of wastewater treatment with H_2 generation for fuel cells should be given more consideration (Angenent *et al*., 2004). Although bioethanol and biodiesel are currently the major targets of biomass energy, hydrogen is still considered the ultimate solution of clean and recyclable energy carrier in a long term (Kapdan and Kargi, 2006).

1.1.2 Mesophilic and Thermophilic Temperature in Anaerobic Digestion

The most important physical factor for achieving successful anaerobic digestion is temperature. In anaerobic digestion there are generally two temperature ranges.

Anaerobic sludge digestion can occur in the mesophilic range (35 °C), which is more usual, or in the thermophilic range (55 °C), which is less common. It is important that the temperature remains constant. Other physical factors, such as mixing, volatile solids loading and hydraulic retention time are also important (Vindis *et al*., 2009).

Mesophilic temperature regimes (30–40 °C) have long been adopted for anaerobic digestion, showing good operational performance. Thermophilic regimes (50 – 60 °C) have also been adopted for anaerobic digestion showing several advantages, such as an increased destruction rate of organic solids, improved solids– liquid separation, and increased destruction of pathogenic organisms (Buhr and Andrews, 1977; Krugel *et al*., 1998; Rimkus *et al*., 1982). However, the use of thermophilic anaerobic digestion has been limited, because of some disadvantages like poor supernatant quality and poor process stability related to chronically high propionate concentrations (Kugelman and Guida, 1989).

It has been shown by previous studies that thermophilic microorganisms are characterized by their higher substrate utilization and growth rates as well as higher decay rate compared to mesophilic bacteria. Studies undertaken by several researchers (Harris and Dague, 1993; Wiegant *et al*., 1986; Zinder *et al*., 1984) showed that thermophilic systems were capable of treating higher organic loadings and had higher specific growth rate as compared to their mesophilic counterparts. The yield of these microorganisms per unit amount of substrate is lower. The lower growth yield of thermophilic bacteria could be due to their increased decay rate, which is double that of mesophilic cultures because the cells have a tendency to lyse quickly under thermophilic

conditions (Speece, 1996), and may be due to their higher energy requirement for maintenance or the specific molecular properties of enzyme reactions at thermophilic temperatures (Zeikus, 1979).

1.1.3. Fundamental Basis for Anaerobic Fluidized Bed Reactor (AFBR).

Several high-rate anaerobic reactors were successfully tested for the biological production of hydrogen. Among the high-rate anaerobic reactors used for biological production of hydrogen is the anaerobic fluidized bed reactor (AFBR). In AFBRs, the microbial film is retained by natural adherence of microorganisms to particles of a solid support medium, which is its most influential variable. (Abreu *et al*., 2009). The anaerobic fluidized bed reactors (AFBR) are treatment systems that take advantage of the principle of fluidization to promote adequate mass transfer between the liquid to be treated and the microorganisms that act to degrade the organic matter. This type of reactor with adhered biofilm has been widely used as a biological treatment system for effluents with high efficiency and short hydraulic retention time (*HRT*) (Lin *et al*., 2009; Barros *et al*., 2010).

Some studies have shown that the products of fermentation depend on the type of substrate used and the operating conditions of the reactor, e.g., the hydraulic retention time (*HRT*), temperature, and pH. In particular, pH has the greatest influence on the composition of the acidogenic reactor effluent. (Ren *et al*., 1997). It influences hydrogen production because it can affect the hydrogenase activity as well as the metabolic pathway (Wang and Wan, 2009). Concisely from several literature, the optimum pH

value for hydrogen production is between 5.5 and 6.0. A pH between 6.0 and 6.5 can produce an excessive amount of propionic acid van Ginkel *et al*. (van Ginkel *et al*., 2001; Fang and Liu, 2002; Li *et al*, 2008; Aceves-Lara *et al*.2008). However, pH values lower than 4.5 are conducive to the production of H_2, CO_2, acetic acid, butyric acid, and ethanol (Ren *et al*., 1997). The literature presents contradictory results in regard to the optimum pH value for hydrogen production. Possible reasons for this lack of consensus are the type of inoculums and substrate used in these studies as well as the pH range under investigation (Wang and Wan, 2009). Contradictory results have been described in the literature. For instance, Barros et al. achieved a high hydrogen yield (*HY*) (1.90 and 2.59 mol H_2 mol^{-1} glucose, respectively) and a low ethanol concentration (1.96 and 4.35 mM, respectively) with glucose as the carbon source, polystyrene and expanded clay as the support materials, and alkalis for pH control (Barros *et al*., 2010). Amorim *et al*. and Shida *et al*. also achieved a high *HY* (2.49 and 2.29 mol H_2 mol^{-1} glucose, respectively) and a low ethanol concentration (1.86 and 1.18 mM, respectively) with glucose as the carbon source and expanded clay as the support material, but without using alkalis (Amorim *et al*., 2009; Shida *et al*., 2009). Abreu *et al*. reported a low *HY* (0.8 mol H_2 mol^{-1} arabinose) and a high ethanol concentration (197.43 mM) with arabinose as the carbon source and a batch reactor and the use of an alkalizing agent for pH control (Abreu *et al*., 2009). However, Wu et al. achieved a high *HY* (1.04 mol H_2 mol^{-1} hexose) and ethanol concentration (20.43 mM) with polyethylene-octane elastomer as the support medium, pH control, and glucose as the carbon source, despite the fact that the production pathways of these biofuels compete with one another (Wu *et al*., 2007).

Although *AFBRs* possess characteristics favourable for the production of gaseous products such as H_2, they have been utilized less frequently for dark H_2 fermentation (Lin *et al*., 2006). Therefore, the present study focused on continuous biohydrogen production in *AFGBs* via mixed-culture biofilms grown on cylindrical activated carbon (*CAC*) support materials. Volumetric hydrogen productivity (*HP*), hydrogen yield (*HY*), specific hydrogen productivity per g of bacterial biomass (*SHP*), biomass densities and hydraulic retention time (*HRT*) were evaluated to quantify biomass and the entire performance of the *AFGB* reactor.

1.2. Research Problem Statement

The main research objective was to develop a thermophilic anaerobic fluidized bed bioreactor system for the production of biohydrogen from sucrose as the carbon source. The central problem of biohydrogen generation is that there appears to be an inverse relationship between volumetric hydrogen productivity and substrate conversion efficiency. Factors that promote hydrogen productivity tend to decrease hydrogen yield. These factors include high organic loading rates and high microbial biomass densities. They also result in an increase in dissolved hydrogen in the bioreactor system which in turn decreases the yield of hydrogen. Factors that promote high yields include high rates of hydrogen stripping via N_2 gas sparging, "low substrate loading rates and low bacterial densities." The latter two factors result in low productivities. The main problem is how to simultaneously achieve high productivities and high yields. A potential solution to this problem would be increasing the rate of degassed effluent recycling through the bioreactor bed. If the rate of degassed effluent recycling was increased above some

critical threshold then would be expected that hydrogen productivity (*HP*) and hydrogen yield (*HY*) also increase.

1.3 Hypothesis

Simultaneous achievement of high *HP* and high *HY* values could be facilitated by the increasing temperature of the bioreactor and by increasing the de-gassed effluent recycle rates through the bioreactor bed.

1.4 Project Objectives

Other aims include the following

- Induction, development and growth of thermophilic bacterial granules.
- Achievement of high volumetric bacterial biomass densities.
- Operation of the bioreactor low *HRTs* and high organic substrate loading rates.
- Operation of the bioreactor at high effluent recycle rates.
- Theoretical energy balance study of the bioreactor system.

Other specific aims under the objectives include the following:

- To successfully promote the growth and development of bacterial granule consisting of a mixed consortium of rumen bacteria.
- To develop a strategy for selective growth of hydrogen producing bacteria.
- To investigate the heat treatment of bacterial mass at different combinations of time and temperature to examine its effect on biohydrogen production.

- To evaluate the process engineering components, e.g. Optimal temperature, pH, biogas measurement, hydraulic retention time (*HRT*) and organic loading rate (*OLR*).
- To estimate granule diameter and interpretation of bacterial features

1.5 Scope of the Project

The scope of the project involved the following range of activities:

- Production of anaerobic thermophilic multispecies inoculum.
- Design and construction of bioreactor systems for biohydrogen production from sucrose.
- Optimization of bioprocess operation for maximization of biohydrogen production.
- Theoretical analysis of the biohydrogen production process.

1.6 Key Research Question/S To Be Answered

- What is the best possible procedure that would maximize optimal mass production of hydrogen at a reduced and affordable cost?
- Would the anaerobic fluidized bed reactor be able to stand the test of time at high temperature?
- What are the factors/parameters that will favour hydrogen production?
- Would the process operating conditions be conducive for granules formation?

- Can thermophilic temperature enhance the production of hydrogen?
- Would the process design be able to create an energy balance model for thermophilic biohydrogen production

1.7 Thesis Outline

The outline of the entire thesis will include:

Chapter one

This chapter discusses the background and motivation of the study, research problem, hypothesis, scope of the project, research questions, purpose and aims and the expected contribution to knowledge.

Chapter two

This chapter, which contains the literature review, will be structured into three parts: The first part gives an overview of hydrogen production and bioenergy from biomass, the second part focuses mainly on fuel cell technology and the last part is on hydrogen economy process and its sustenance with regards to application.

Chapter three

This chapter explains the influence of bacterial biomass density on hydrogen production efficiency in a thermophilic granular bed bioreactor.

Chapter four

This chapter dwells on the stability of biohydrogen production at extreme thermophilic (70 ^{o}C) temperatures by an undefined bacterial culture

Chapter five

The chapter describes biohydrogen production by bacterial granules adapted to grow at different thermophilic temperatures

Chapter six

This chapter shows the Interrelationships between bioreactor volume, effluent recycle rate, temperature, pH, $\%H_2$, hydrogen productivity and hydrogen yield with undefined bacterial cultures.

Chapter seven

This chapter concludes all achieved aims and objectives of the research

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Global energy consumption due to industrial development and human activities is rising significantly, and is likely to continue accelerating; however, fossil fuels are still one of the biggest energy contributors (Reese S. Thompson, 2008; Hallenbeck, 2011; Tae-Hyeong *et al*., 2012). It is estimated that the global power supply is still based on 84.8% fossil energy (Zurawski *et al*. 2005). Nonetheless, by virtue of its unrivaled environmental benefits, H_2 is widely considered to be the energy carrier of the future (Benemann, 1996, Zuttel *et al*., 2008 and Jones, 2008). H_2 can be generated via a number of established technologies, including renewable biological routes (Nath and Das, 2003, Claassen *et al*., 1999). Strategies for H_2 production from plant sources essentially follow two major routes: the photochemical conversion of sunlight or dark fermentative processes. Although many scientific issues still remain to be understood, the fermentative path currently appears to be closer to practical utilization. The benefits of this approach include the low cost of biomass, and the fact that byproducts of agricultural food production can be used as feedstocks in the processes (Fan *et al*.,2006, Pattra *et al*., 2008 and Kyazze *et al*., 2008).

Research on biological H_2 production has been carried out for over a quarter century (Hallenbeck and Benemann, 2002; Hallenbeck, 2011) and a wide variety of biological H_2-production processes have been investigated, including direct biophotolysis, indirect biophotolysis, photo-fermentations and dark fermentation. Anaerobic dark-fermentation

systems offer the best potential for practical application (Levin *et al*., 2004). Some basic advantages over other processes (Levin *et al*., 2004; Benemann, 1996; Nandi and Sengupta, 1998).include:

- Better process economy for lower energy requirements, process simplicity, higher rates of H_2 production and utilization of low-value waste as raw materials (Levin *et al*., 2004; Benemann, 1996; Nandi and Sengupta, 1998)

- More effective pathogen removal (Bendixen, 1994; Lund *et al*., 1996; Sahlstrom, 2003). This is especially true for multi-stage digesters (Kunte *et al*., 2004; Sahlstrom, 2003) or if a pasteurization step is included in the process.

- Minimal odour emissions as 99% of volatile compounds are oxidatively decomposed upon combustion, e.g. H_2S forms SO_2 (Smet *et al*., 1999).

- The slurry produced (digestate) is an improved fertiliser in terms of both its availability to plants (Tafdrup, 1995) and its rheology (Pain and Hepherd, 1985).

- Successful in treating wet wastes of less than 40% dry matter (Mata-Alvarez, 2002).

2.2. Biohydrogen as Renewable Energy

Interest in biohydrogen started getting prominence in early 90s, when it became apparent that atmospheric pollution by fossil fuels is not only unhealthy locally, but might also cause significant climate changes globally. As a result, biological hydrogen production became a focus of Governmental support, particularly in Germany, the United States (U.S) and Japan, with meager efforts in the other countries. Biological hydrogen production now has gained such a tremendous impetus that more than 30 countries have chosen to invest in the concerned research (Benemann, 1996, Momirlan *et al*., 2002). Realization of practical processes for biohydrogen production would result in a major, novel biological source of sustainable and renewable energy, without greenhouse gas emissions or environmental pollution. For H_2 to be renewable, it must come from renewable sources hence the focus on biomass.

Biomass is the fourth largest source of energy in the world, accounting for about 15%of the world's primary energy consumption and about 38% of the primary energy consumption in developing countries (Chen *et al*., 2003). Biomass resources can be divided into two broad categories; natural and derived materials. These categories can be further subdivided as wastes, forest products and energy crops (Narvaez *et al*., 1996) There is a vital need of a process, which can convert biomass into useful energy products like oil, gases, etc. Pyrolysis and gasification of the waste materials have been found to be the most favourable thermo-chemical conversion processes for utilizing renewable biomass energy (Goyal *et al*., 2006).

Biomass has always been a major source of energy for mankind from ancient times. Presently, it contributes around 10–14% of the world's energy supply (Putun *et al*., 2001). Biomass has been identified as an important source for alternative fuels and added-value chemicals through an annual production of up to 1.7–2.0×1011 tons, (Klass, 1998, Huber et al., 2006; Kamm *et al*., 2006). Currently, biomass contributes about 12% of today's world energy supply, while in many developing countries it contributes 40–50% energy supply (Demirbas, 2001). Biomass research is recently receiving increasing attention because of the probable waste-to-energy application. For instance, 150 GT of vegetable bio-matter generated globally every year can produce about 1.08 -1010 GJ energy (Larminie *et al*., 2000).

2.3. Conventional Methods on H_2 Production in Comparison to Biohydrogen Methods.

At present hydrogen is produced mainly from fossil fuels, biomass and water. The methods of hydrogen production from fossil fuels are: steam reforming of natural gas, thermal cracking of natural gas, partial oxidation of heavier than naphtha hydrocarbons and coal gasification. Methods of hydrogen production from water are: electrolysis, photolysis, thermochemical process and direct thermal decomposition or thermolysis. However, methods of hydrogen production from biomass are: pyrolysis or gasification (Mormirlan and Veziroglu, 1999; Mckendry, 2002; Demirbas, 2004)

From the above listed processes, nearly 90% of hydrogen is produced by the reactions of natural gas or light oil fractions with steam at high temperatures (steam reforming). The steam methane reforming process can be used industrially to produce hydrogen, carbon

monoxide and their mixtures. Depending on the quantities of the desired products, the elements of the process can be adapted. In its simplest form, the steam methane reforming process for pure hydrogen production consists of four stages as shown in Figure 2.1: a desulphurization unit, a steam methane reformer, shift reactor(s), and finally pressure swing adsorption (Nazim, 2009). It is of great advantage that the process generates few wastes, chemical storage, or liquid effluents; none of these are particularly hazardous (Walter, 1993). More so, the presence of a centralized waste water treatment facility on the refinery site further limits the impact on the aquatic environment.

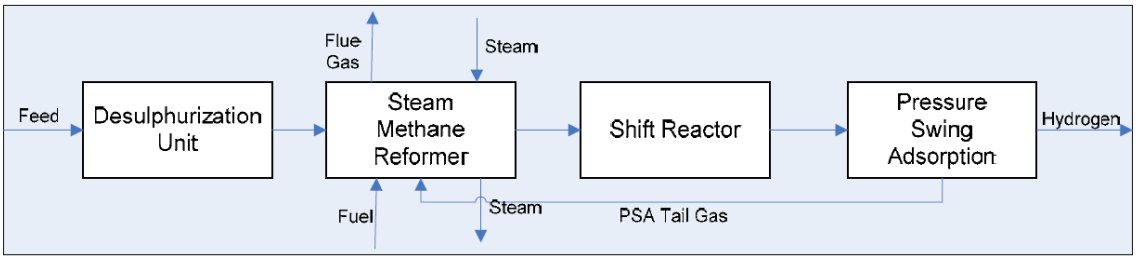


Figure 2.1: Hydrogen Production by Steam Methane Reforming

These industrial methods mainly consume fossil fuel as energy source, and sometimes hydroelectricity (Rosen *et al*., 1998, Casper, 1978). However, both thermochemical and electrochemical hydrogen generation processes are energy intensive and not always environment-friendly. 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 they also open a new avenue for the utilization of renewable energy resources which are inexhaustible (Benemann, 1997, Tanisho *et al*., 1983). In addition, they can also use various waste materials which facilitate waste recycling.

2.3.1. Biological Hydrogen Production

Biological production of hydrogen (biohydrogen), using (micro) organisms, is an exciting new area of technological development that offers the potential production of usable hydrogen from a variety of renewable resources. Biological systems provide a wide range of approaches to generate hydrogen, and include direct biophotolysis, indirect biophotolysis, photo-fermentations, and dark-fermentation (Dunn, .2002; Das *et al*., 2001; Hallenbeck *et al*., 2002, 2012 and Nandi *et al*., 1998).

Anaerobic fermentation route is a promising biological process for hydrogen production owing to the fact that hydrogen can be produced continuously at high rate from renewable organic compounds (Benemann, 1996). However, biological methods depend on hydrogenases that catalyse the reaction $2H^+ + 2e^- \leftrightarrow H_2$ (g) (Evans and Pickett, 2003). Hydrogen gas may be produced through either photosynthetic or fermentation processes, but fermentative hydrogen production is more efficient than photosynthetic ones (Yoshida *et al*., 2005).

Over the past two decades, anaerobic hydrogen fermentation has attracted worldwide attention (Rachman *et al*., 1998; Zhang *et al*., 2006). This is largely due to the soaring concerns on environmental deterioration and sustainability derived from the utilization of conventional fossil fuels and on the potential of hydrogen as an ideal alternative. Currently, laboratory-scale studies on anaerobic hydrogen fermentation technology are being conducted by a large number of research groups in different countries across the world (Kim *et al*., 2004; Lin *et al*., 2003).

2.3.2 Substrate for Hydrogen Production

Cellulose is the most plentiful biopolymer on earth and it is the principal component of plant biomass. Plant cell walls contain lignocellulose, which is composed of three major polymers: cellulose, hemicellulose, and lignin (Weimer and Zeikus, 1977; Haug, 1993). Cellulose fibers have a partial crystalline structure, integrated with hemicellulose and embedded in a lignin matrix (Claassen *et al*., 1999). The conversion of cellulose to hydrogen by microbial fermentation, therefore, represents a partial answer to waste accumulation, the depletion of hydrocarbon fuel reserves, and carbon dioxide release (Ueno *et al*., 1995; Zeikus, 1980). Anaerobic biohydrogen production can be divided into two main categories: photosynthetic bacteria and chemotrophic bacteria. The difference between both is that the latter has the ability to the difference between both is that the latter has the ability to generate hydrogen without photoenergy (Gray and Gest, 1965; Liessens and Verstraete, 1986). Cellulose can act as substrate for hydrogen production by anaerobic heterotrophic fermentation (Guedon *et al*., 1999). Hydrogen is a major intermediate in anaerobic fermentation of cellulose by fibrolytic and fermentative microorganisms (Giallo *et al*., 1985). Some researchers also have introduced natural anaerobic microorganisms, obtained from anaerobic digested sludge and sludge compost, to generate biohydrogen from cellulose by mixed batch cultures (Ueno *et al*., 1995; Fond *et al*., 1983). However, in many ecosystems where lignocellulose compounds are degraded, interspecies hydrogen transfer occurs and the hydrogen produced by cellulolytic bacteria is used immediately by methanogens, sulfate reducers, and acetogens (Wolin and Miller, 1988; Morvan *et al*., 1996). If the activity of hydrogentrophic bacteria contained in anaerobic digested sludge were inhibited, the sludge would possess significant capacity to transform cellulose into hydrogen gas

2.4. Factors Affecting Biohydrogen Fermentation Process

2.4.1. Temperature

Temperature is one of the most relevant factors influencing biological H_2 fermentation process, substrate degradation, product distribution and bacterial growth rate. Infact, the entire H_2 production process. In a fermentative H_2 producing reactor, the microbial community is also greatly influenced by temperature (Fang *et al*., 2002; Ueno *et al*., 2001). The isolation and identification of fermentative hydrogen producers with a high yield and high production rate of hydrogen are important for development of commercial sustainable biohydrogen production process. Various hydrogen producing strains including species of enterobacter spp. (Fabiano and Perego, 2002), clostridium spp. (Taguchi *et al*., 1995) and bacillus spp. (Kotay and Das, 2007) have been identified and studied. Among the hydrogen producers, mesophilic bacteria have been studied most extensively. Normally it is observed that hydrogen production yield of 1-2 mol H_2 mol $^{-1}$ hexose are obtained with mesophiles, while thermophiles displays a yield higher than 2 mol H_2 mol $^{-1}$ hexose (Van Niel *et al*., 2002). Hydrogen yields can be improved by increasing hydrogen production through acetate end product reaction, and decreasing or preventing butyrate, ethanol and propionate product reaction. One way to accomplish this is through fermentation process with thermophiles or extreme thermophiles, operating at temperatures higher than 60 ºC (Van Niel *et al*., 2002 and Kadar *et al*.,2004). Thus, higher temperature is more feasible for the conversion reaction toward hydrogen due to favorable thermodynamics conditions. Thermophilic bacteria are therefore considered as most promising microorganisms than mesophilic bacteria for hydrogen production (Sommer *et al*., 2003).

However, research at extreme-thermophilic temperatures gain increasing interest because the hydrogen production yield is much higher than the ones in mesophilic and thermophilic temperatures (Reith *et al*. 2003). In addition, extreme-thermophilic conditions result in higher hydrolysis activity, which is the bottleneck for degradation of complex substrates such as manure and household solid waste (Hartmann and Ahring 2005). Moreover, extreme-thermophilic conditions have the advantage of better sanitation and lesser contamination chance from methanogens (Kotsopoulos *et al*. 2006).

Hence, the operating conditions of this study will focus on thermophilic and extreme-thermophilic temperatures, because within this temperature range, bacteria are able to utilize a wide range of organic waste (Noike and Mizuno, 2000). Theoretically, the maximum hydrogen production yield could be obtained by thermophilic (50 – 55 ºC) bacteria or extreme thermophilic (55 – 80 ºC) bacteria (Van Niel *et al*., 2002).

2.4.2. pH

The rate of hydrogen evolution from an anaerobic fermentation was dependent on the pH, loading rate, biogas circulation and hydraulic retention time (*HRT*) for the acidogenic phase (Fang and Liu, 2001; Noike and Mizuno, 2000; Tanisho, 1998). These parameters are used mostly to control the operation by blocking the methanogenesis of the anaerobic pathways. However, methanogenesis is the critical stage in anaerobic bio-hydrogen production process where there is a rapid rate of H_2 consumption by methanogens.

Besides, pH plays an important role in the H_2-producing process, as it has a significant influence on the hydrogenase activity and on the metabolism pathways, e.g., utilization of carbon and energy sources, efficiency of substrate degradation, synthesis of proteins and

various types of storage material and release of metabolic products from cells (Baily *et al*., 1986). Moreover, pH variation can affect cell morphology and structure and, therefore, flocculation and adhesion phenomena (Gottschalk., 1986). If pH is not maintained in the desired range, it could inhibit H_2 production or cause a microbial population shift, resulting in cessation of H_2 production (Van Ginkel *et al*., 2001). A considerable number of studies have been carried out to evaluate the effects of pH on H_2 production in various types of reactors, such as continuously stirred tank reactor (CSTR) and trickling biofilter (Kim *et al*., 2004; Oh *et al*., 2004).

However, to narrow it down to the present study, were rumen bacteria is the main focus, the rumen, although well buffered by bicarbonate, phosphate, protein, and volatile fatty acids, can vary in pH from approximately 7.0 to less than 5.0 under different dietary conditions. In vivo (Mackie and Gilchrist, 1978) and in vitro (Hobson and Summers, 1965) observations have indicated that the relative success of rumen bacteria is correlated with pH, and the work of Russell et al, shown that an acidic environment can decrease the maximum growth rate of rumen bacteria (Russell *et al*., 1979). As the ruminant animal is largely dependent on microbes as a protein source, the efficiency of rumen microbial growth is of critical importance to ruminant performance. Conclusively, in this study, the operation temperature and pH of H_2 production is selected as the target operation parameter because it usually markedly affects the growth rate and metabolic activity of microorganisms in the mixed consortium.

2.4.3. Organic Loading Rate (OLR)

Organic loading rate (OLR) is an important parameter for continuously producing hydrogen in the bioreactors. In order to optimize a system for hydrogen production, it is essential to define either a range of the OLR that the system can handle effectively, or optimal OLR for a maximum hydrogen yield. However, from the literature search, there is no clear relationship between the hydrogen yield and the OLR. In some cases high OLR decreased the hydrogen yield (Van Ginkel and Logan, 2005) whereas in some others high OLR increased the hydrogen yield. (Zhang *et al*., 2004) For waste activated sludge as a seed material, it appears that increasing the OLR within the ranges of 40–160 g-COD/L-d increased hydrogen yield in which the optimum yield of 1.6 mol H_2/mol glucose was obtained at an OLR of 120 g-COD/L-d (Wu *et al*., 2006) However, the hydrogen yield was found to decrease with an increase in OLR when anaerobically digested sludge (Kyazze *et al*., 2006) and soil microorganisms (Van Ginkel and Logan, 2005) were used as the inoculums. Although lower molar hydrogen yields at higher OLR have been attributed to the inhibitory effect of higher hydrogen partial pressure in the growth medium, (Van Ginkel and Logan, 2005; Ruzicka, 1996) variations in the composition of bacterial communities that become established at different OLR (Luo *et al*., 2008) may be a major reason for lower yields.

2.4.4. Hydraulic Retention Time

HRT is also an important parameter for dark fermentation process. In continuously stirred tank reactor (CSTR) system, short *HRTs* were used to wash out the slow growing methanogens and select for the acid producing bacteria (Chen *et al*., 2001), while too

short *HRT* could lead to bad hydrolysis of organic wastes (Han and Shin 2004). Normally, in an anaerobic process, pH and *HRT* are coupled parameters: short *HRT* results in low pH. Both pH and *HRT* have been demonstrated as the effective ways to separate hydrogen producing bacteria and hydrogen consuming archaea at mesophilic and thermophilic conditions (Oh *et al*., 2004). However, effects of pH and *HRT* are interrelated that no dedicated research has isolated the effect of these two parameters separately.

2.4.5. Hydrogen Partial Pressure

H_2 partial pressure is key parameter in the production of hydrogen by fermentative bacteria (Angenent *et al*., 2004), but difficult to control. The hydrogen concentration in the liquid phase, related to hydrogen partial pressure, is one of the key factors affecting the hydrogen production (Hawkes *et al*., 2002). The partial pressure of H_2 (pH_2) is an extremely important factor especially for continuous H_2 synthesis (Hawkes *et al*., 2007). Hydrogen synthesis pathways are sensitive to H_2 concentrations and are subject to end-product inhibition. As H_2 concentrations increase, H_2 synthesis decreases and metabolic pathways shift to production of more reduced substrates such as lactate, ethanol, acetone, butanol, or alanine. As the temperature increases, however, conditions that favor hydrogen formation reactions are less affected by H_2 concentration (Tamagnini *et al*., 2002). Continuous H_2 synthesis requires pH_2 of 50 kPa at 60°C (Lee and Zinder, 1988), 20 kPa at70°C(van Niel *et al*., 2002), and 2 kPa at 98°C under standard conditons (Adams, 1990; Levin *et al*., 2004).

2.5. Microbiology of Biohydrogen Production

Basic research on microbiological hydrogen production processes were undertaken in the twenties and applied in the seventies of the 20th century. Although these were mainly focused on photosynthesis systems, among the microbiological methods of hydrogen production besides water biophotolysis (microalgae) and photofermentation (photosynthesizing bacteria) one can also distinguish a very promising in terms of commercial implementation, dark anaerobic fermentation (anaerobic heterotrophic bacteria) (Claassen *et al*.,2000, 2006). Applying biological methods of hydrogen production in fuel cells supply systems (Claassen *et al*.,2000, Levin *et al*., 2004 & Duerr *et al*., 2007) as well as organic waste-based hydrogen production as a fuel for transport, heating and electricity generation systems (Claassen *et al*.,2000, Angenent *et al*., 2004 and van Ginkel *et al*., 2005) are seriously considered, notwithstanding a commercial scale installation of these types are still missing.

Nature in the form of microorganisms has been using hydrogen as a primary fuel source for billions of years, and has solved the problem of converting hydrogen to electricity by means of the biocatalyst, hydrogenase (Table 2.1). Although they catalyze a deceptively simple reaction, they have proven to be some of the most complex and ingenious bioinorganic structures known. Microorganisms generate hydrogen for two principle reasons: to dispose of excess reducing equivalents and as a by-product in nitrogen fixation. Microbial hydrogen production is an attractive process for supplying a significant share of the hydrogen required for the near future.

Table 2.1: Biochemical diversity of biohydrogen production

Heterotrophic	Photoheterotrophic
Fermentative H_2 production from biomass by heterotrophic bacteria ($C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$) H_2 production from CO by photosynthetic bacteria ($CO + H_2O \rightarrow H_2 + CO_2$)	Biophotolytic H_2 production by green algae or cyanobacteria (water splitting) ($12H_2O \rightarrow 12H_2 + 6O_2$) Photoproduction of H_2 from biomass by phototrophic bacteria ($C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$)

In great quantities, microbial species, belonging to the genera enterobacter, citrobacter, bacillus, and clostridium have been reported to produce hydrogen through dark fermentation (Nandi *et al*., 1998). Apart from pure cultures, various mixed micro-flora and co-cultures have also been explored for hydrogen production from carbohydrates (Nandi *et al*., 1998; Das *et al*., 2001; Levin *et al*., 2004). Nevertheless, the quest for ideal microbe(s) for microbial H_2 production has thrust the researchers to screen various sources.

2.6. The biochemistry of anaerobic degradation of complex organic matter

Anaerobic fermentation is a process of substrate molecule (organic compounds) degradation and transformation, during which one of the products is oxidized and the other one is reduced as follows: carbohydrates → organic acids + H_2 + CO_2 (Kunicki-Goldfinger, 1994).

In order to hydrolyze these particulate organics, which are mainly proteins, carbohydrates and lipids microorganisms synthesize and secrete various hydrolyzing enzymes (Nielsen *et al*., 1992; Raunkjaer *et al*., 1994). The degradation of complex organic matter has been described as a "multi-step process of a series of parallel reactions" (Pavlostathis and Giraldo-Gomez, 1991), which is accomplished by a complex microbial community

involving hydrolytic, fermentating, homoacetogenic, syntrophic and methanogenic microorganisms (Zinder, 1993; Stams, 1994; Schink, 1997). Biodegradation of carbohydrates, proteins, and lipids is carried out sequentially by several physiological groups of anaerobic bacteria that work together (Figure 2.2). The process can be described by the following four steps:

Hydrolysis: It is the first stage of anaerobic digestion were extracellular enzymes produced by the inhabiting hydrolytic and fermentative bacteria hydrolyze the macromolecules into smaller and more digestible forms and ferment the resulting sugars to carboxylic acids and alcohols. The non-methanogenic microorganism responsible for hydrolysis and fermentation are facultative and obligate anaerobic bacteria (Metcalf & Eddy, 2003). The hydrolytic activity is of significant importance in high organic waste and may become rate limiting during the anaerobic digestion of wastewater rich in organic solids (Valentini *et al*., 1997). Some industrial operations overcome this limitation by the use of chemical reagents to enhance hydrolysis. The application of chemicals to enhance the first step has been found to result in a shorter digestion time and provide a higher methane yield (RISE-AT, 1998).

Acidogenesis: This includes fermentation and anaerobic oxidation which are executed by a large group of facultative and obligate anaerobes such as *Clostridium*, *Bifidobacterium*, *Desulphovibrio*, *Actinomyces*, and *Staphylococococcus*. Volatile fatty acids (VFA), such as propionic acid and butyric acid are produced along with carbon dioxide and hydrogen. Acidogens have a lower pH optima around 6 (Sanchez *et al*., 2000). However, the

hydrogen content in the reactor is the key factor for regulating the acidogenesis (Harper and Pohaland, 1986).

Acetogenesis: acetogenic bacteria that breakdown volatile acids and alcohols to acetate, hydrogen and carbon dioxide. It's pH optimum ranges at approximately 7 (Sanchez *et al*., 2000)

Methanogenesis: in this process, methanogenic bacteria such as *Methanobacillus*, *Methanococcus, Methanobacterium* and *Methanosarcina* are responsible for converting the end products of the acetogenic reactions to methane gas and carbon dioxide (Metcalf and Eddy, 1991). The required pH range for methanogenesis is between 6.6 and 7.6 with an optimum range between 7.0 and 7.2 (Ghaly, 1996). Methane production is higher from reduction of carbon dioxide but limited hydrogen concentration in digesters results in that the acetate reaction is the primary producer of methane (Omstead *et al*, 1980).

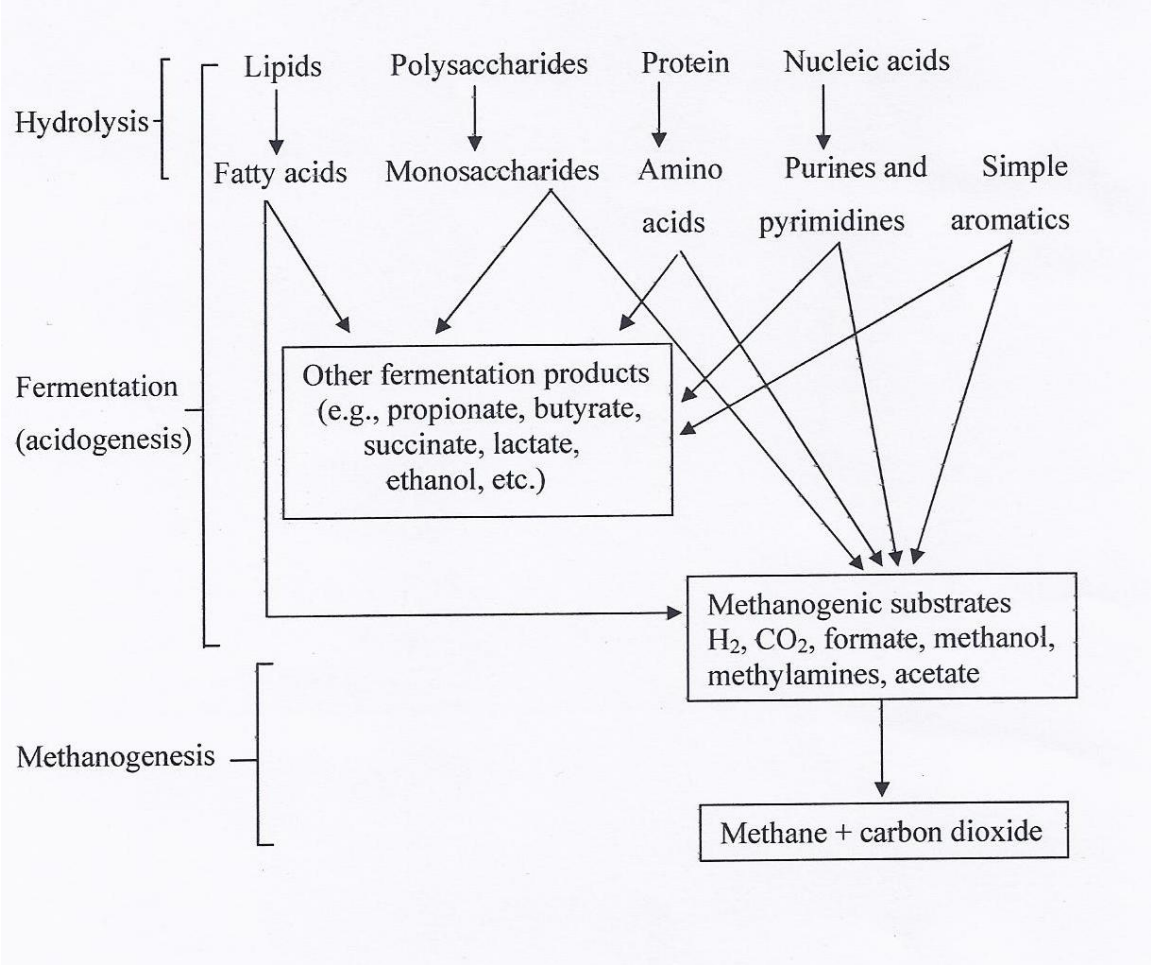


Figure 2.2: Anaerobic process schematic of hydrolysis, fermentation (acidogenesis) and methanogenesis (Metcalf & Eddy, 2003)

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2.7. Evaluation of Hydrogen Productivity on reactor type

Hydrogen can be produced substantially at a high rate if the correct reactor configuration is used in it production process. The hydrogen production rate (HPR) has been considered as an important index to evaluate the performance of continuous hydrogen-producing processes (Chang *et al*., 2002). With regard to the continuous mode operation in hydrogen production, various types of reactors were employed (Chang *et al*., 2002; Lee *et al*., 2007; Li *et al*., 2007; Mohan *et al*., 2007; Ren *et al*., 2002; Zhang *et al*., 2007)., among which continuously stirred tank reactor (CSTR) was mostly reported (Chen and

Lin, 2003; Ren *et al*., 2007; Yu *et al*., 2003). Although stirring operation could improve mass transfer efficiency, the typical suspended-cell systems usually have the potential problems of significantly biomass washout due to high organic loading rate (OLR). While a high OLR was found to be a critical factor to ensure high effective hydrogen productivity. More so, the continuous stirred tank reactor (CSTR) process usually exhibits poor performance in HPR since it is unable to maintain high levels of hydrogen-producing biomass at a short hydraulic retention time (*HRT*) due to its intrinsic structure. (Chen and Lin, 2003; Yu *et al*., 2003) However, immobilized-cell systems have become common alternatives to suspended-cell systems in continuous operations since they are more efficient in maintaining higher biomass concentration, solid/liquid separation and can be operated at high dilution rates (or low retention times) without encountering washout of cells. (Chang *et al*., 2002; Wu *et al*., 2002).

Different anaerobic wastewater treatment systems have been developed over the years around the world including the Anaerobic Filter (AT) (Young and McCarty, 1969), the Upflow Anaerobic Sludge Blanket (UASB) (Lettinga *et al*., 1980), the Fluidized and Expanded Bed Reactor (FEBR) (Schwitzenbaum and Jewell, 1980), the Down Flow Stationary Fixed Film Reactor (DFSFFR) (Murray and van den Berg, 1981) and the Baffled Reactors (BR) (Barber and Stuckey, 1999). Lettinga and co-workers introduced modified versions of the UASB viz. the UASB-septic tank reactor (Bogte *et al*., 1993), the Hydrolysis Upflow Sludge Blanket (HUSB) (Wang, 1994), the Staged Multi-Phase Anaerobic (SMPA) reactor (Lier, 1995) and the two stage-Anaerobic Filter (AF) - Anaerobic Hybrid (AH) system (Elmitwalli *et al*., 2002). Other interesting reactor

configurations that have been investigated include: the Fluidised Bed Reactor (FBR), Anaerobic Baffled Reactor (ABR) (Foresti, 2001), Horizontal-Flow Anaerobic Immobilised Biomass (HAIB) (Zaiat *et al*., 2000) and Sequencing Batch Reactor (SBR) (Cybis and Pescado, 2000; Callado and Foresti, 2001).

Cell immobilization approaches (mostly fixed-film or granular-sludge systems) have been applied to produce H_2 continuously in fixed-bed (or packed-bed) bioreactors (Chang *et al*., 2002; Kumar and Das, 2001; Lee *et al*., 2003; Tanisho and Ishiwata, 1995; Palazzi *et al*., 2000; Rachman *et al*.,1998; Yokoi *et al*., 2002) granular-sludge bed bioreactors (e.g., CIGSB) (Fang *et al*., 2002; Liu and Fang, 2002; Lee *et al*., 2004a&b), trickling biofilter reactors (TBR) (Oh *et al*., 2004), and up-flow anaerobic sludge blanket reactor (UASB) (Chang and Lin, 2004; Yu *et al*., 2002). Although fluidized bed reactors (FBRs) possess favourable characteristics for the production of gaseous products, like H_2, they have not been widely applied for fermentative H_2 production (Wu *et al*., 2003; Guwy *et al*.,1997).

Interest in AFBR (anaerobic fluidized bed reactor) has grown as it combines the recovery of usable energy with good process efficiency and stability. Potential AFBR applications for the treatment of hazardous waste with inhibitory/recalcitrant compositions have also been reported (Seckler *et al*., 1996; Lin *et al*., 1998; Schreyer and Coughlin, 1999; Hansen *et al*., 1998; van Lier *et al*., 2001; Rodrı´guez-Cano, 2003). Hence, in this study, a modified anaerobic fluidized granular bed bioreactor (*AFGB*) was utilized The *AFGB* were designed and operated under varied parameters like; pH, OLR, HRT using sucrose

as substrate and under themophilic temperatures (55–70 °C) in order to assess the H_2-producing ability under different operating conditions.

2.8. Hydrogen and Fuel Cell Technology

Fuel cells are electrochemical devices that convert the chemical energy of reactants (both fuel and oxidant) directly into electrical energy (Xianguo, 2006). The direct chemical conversion into electricity and heat does not involve combustion cycles. Therefore, the use of the thermal-mechanical-electric sequence with Carnot‟s theorem limitation in the conventional indirect technology is avoided (Kordesh and Simader, 1996). Although heat engines and fuel cells are both energy conversion devices that require reactants being stored externally, fuel cells on the other hand have the overall efficiency to produce profitable energy which is about twice that obtainable by means of conventional combustion engines (Alcaide *et al*, 2006). This is because, the operation of fuel cells at a known temperature generates electrical energy by electrochemical process of the reactants in one step without any intermediate form of energy.

The prospect of converting hydrogen into electricity via fuel cells makes the application of hydrogen energy very promising (Moore *et al*., 1998). The major merits of hydrogen are its ability to be stored compactly as a metallic hydride and the production of water as the only by-product resulting from its combustion (Billings, 1991)

. Its uses are: it acts as a reactant in hydrogenation processes as well as produce lower molecular weight compound, crack hydrocarbons or remove sulfur and nitrogen compounds. Also, it is an O_2 scavenger and can be used to chemically remove trace

amount of O_2 to prevent oxidation and corrosion. Finally, it can serve as a coolant in electrical generators.

Major efforts are devoted to developing alternative electricity production methods. New electricity production from renewable resources without a net carbon dioxide emission is much desired (Lovley, 2006, Davis and Higson, 2007). A technology using microbial fuel cells (MFCs) that convert the energy stored in chemical bonds in organic compounds to electrical energy achieved through the catalytic reactions by microorganisms has generated considerable interests among academic researchers in recent years (Allen and Bennetto, 1993; Gil *et al*., 2003; Moon *et al*., 2006; Choi *et al*., 2003). Bacteria can be used in MFCs to generate electricity while accomplishing the biodegradation of organic matters or wastes (Park and Zeikus, 2000; Oh and Logan, 2005). Figure 2.3 shows a schematic diagram of a typical MFC for producing electricity. It consists of anodic and cathodic chambers partitioned by a proton exchange membrane (PEM) (Wilkinson, 2000; Gil *et al*., 2003).

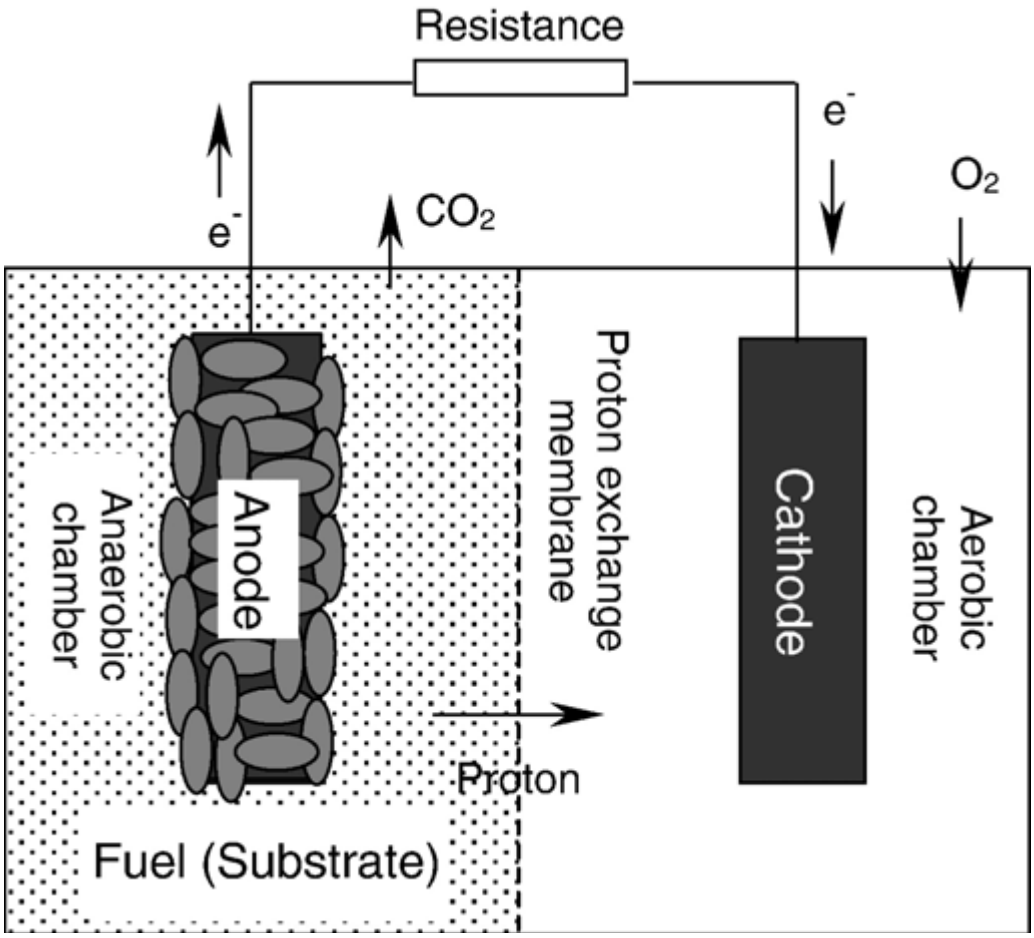


Figure 2.3: Schematic diagram of a typical two-chamber microbial fuel cell.

Hydrogen fuel cells and related hydrogen technologies provide the essential link between renewable energy sources and sustainable energy services (Levin *et al.*, 2004) The transition from a fossil fuel-based economy to a hydrogen energy-based economy, however, is fraught with many technical challenges, from the production of sufficient quantities of hydrogen to its storage, transmission and distribution. (Dunn, 2002). One of the major precincts to the practical application of biohydrogen systems is that scientists who study biohydrogen systems do not talk to engineers who develop hydrogen fuel cell technologies (and vice versa). Thus, the rates of hydrogen produced by biological systems are unknown to fuel cell engineers and the amounts of H_2 required for practical

applications, such as fuel cells, are unknown to biohydrogen researchers. Moreover, the rates of hydrogen produced by the various biohydrogen systems are expressed in different units, making it difficult to assess and compare the rates and amounts of hydrogen synthesized by different biohydrogen technologies. Rates of H_2 production are reported variously as ml of H_2/ml of culture/h, l of H_2/l of culture/h, µmol of H_2/l of culture/h, nmol of H_2/µg of protein in the culture/h, or µmol of H_2/mg of chlorophyll (chl) a/h (Levin *et al*., 2004).

In order to assess the potential application of the various biohydrogen systems, Levin et al calculated the size of bioreactors that would be required to supply sufficient H_2 to proton exchange membrane fuel cells(PEMFC) to generate enough electricity to meet the energy demands of a typical house located in the Pacific Northwest of North America (British Columbia, Canada). The choice of a PEMFC is based on the idea that biohydrogen systems might best be used as a means of delivering small, distributed power systems to communities.

In British Columbia, the average non-electrically heated house uses 12,971 kWh of electricity every year, while an electrically heated house requires 19,606 kWh (BC Hydro, unpublished data). This amount of energy could be produced by PEMFCs with output rating sequal to the average electrical loads, approximately 1.5 and 2:5 kW, respectively (Table 2.2).

Table 2.2: The energy produced by a range of fuel cell sizes as a percentage of coastal residential loads in British Columbia, Canada

Fuel cell Output power kW	Yearly energy production kWh	Percentage of BC Hydro load	
		Non-Electrically Heated house (%)	Electrically heated house (%)
0.25	2190	17	11
0.50	4380	34	22
0.75	6570	51	34
1.00	8760	68	45
1.50	13,140	101	67
2.00	17,520	135	89
2.50	21,900	169	112
3.00	26,280	203	134
4.00	35,040	270	179
4.60	40,296	311	206
5.00	43,800	338	223

Alternatively, the size of the fuel cells could be increased so that sufficient electricity is generated to meet peak load demands. A 5 kW PEMFC would provide sufficient power to meet the peak load demand of an electrically heated house without difficulty, but would generate excess energy during non-peak load periods. This could create an interesting scenario in which a residential fuel cell unit, fuelled by hydrogen produced biologically, could have potential as a small-scale distributed power generator. Several utilities in the US are beginning to offer "net-metering" programs that allow customers to sell unused electricity (from residential solar installations or electric vehicles, for instance) back to the grid. (Kempton *et al*., 2001)

According to Levin et al, the flow rates of H_2 required to power PEMFCs of various sizes were calculated and are presented in Table 2.3

Table 2.3: Flow rates of H_2 required to power PEM fuel cells

Size of PEMFC (kW)	H_2 flow rate required		
	(g/h)	(mol/h)	(SL/h)
1.0	49	23.9	577.7
1.5	73	35.9	866.6
2.5	121	59.9	1444.3
5.0	243	119.7	2888.5

50% efficiency, 95% H_2 utilization rate, average cell voltage 0.779 V. (Levin *et al*., 2004)

2.8.1 Rates of Biohydrogen Synthesis

A comparison of H_2 production rates reported for several biohydrogen systems is presented in Table 2.4. Conversion of reported units of H_2 production to the standardized unit (mmol H_2/(l × h) reveals the wide range of H_2 synthesis by different biohydrogen systems. Light-dependent biohydrogen systems (direct photolysis, indirect photolysis, and photo-fermentation) all have rates of H_2 synthesis well below 1 mmol H_2/(l×h). dark-fermentation systems, all produce H_2 at rates that are well above 1 mmol H_2/(l×h). The rates of H_2 synthesis by an undefined consortium of thermophilic *Clostridium* (Ueno *et al*., 1996) and by the extreme thermophilic *Caldicellulosiruptor saccharolyticus* (van Niel *et al*., 2002 a&b) are very similar (8.2 and 8:4 mmol H_2/(l × h), respectively). A pure strain of mesophilic *Clostridium* (Taguchi *et al*., 1996) demonstrated very good rates of H_2 synthesis with xylose as a substrate (21.0 mmol H_2/(l×h), and two dark-fermentation systems that utilized undefined consortia of mesophilic bacteria (Chang *et al*., 2002; Lay, 2000; Lay, 2001) had impressively higher rates of H_2 synthesis (64.5 and 121:0 mmol/H_2/(l × h), respectively).

Table 2.4: Comparison of the rates of H_2 synthesis by different technologies

$BioH_2$ System	H_2 synthesis rate (reported units)	H_2 synthesis rate (converted units)	References
Direct Photolysis	4.67 mmol H_2/l/80 h	0.07 mmol H2/(l × h)	Francou and Viginais, 1984
Indirect photolysis	12.6 nmol H_2/µg protein/h	0.355 mmol H_2/(l × h)	Taguchi *et al*., 1994
Photo-fermentation	4.0 ml H_2/ml/h	0.16 mmol H_2/(l × h)	Melis, 2002; Polle *et al*., 2002
CO-oxidation by R. *gelatinosus*	0.8 mmol H_2/g cdw/min	96.0 mmol H_2/(l × h)	Zhu *et al*., 2002
Dark-fermentations			
Mesophilic, pure strain[a]	21.0 mmol H_2/1 l/h	21:0 mmol H_2/(l × h)	Ueno *et al*., 1996
Mesophilic, undefined[b]	1,600.0 l H_2/m3/h	64:5 mmol H_2/(l × h)	Jouanneau and Viginais, 1984
Mesophilic, undefined	3.0 l H_2/l/h	121:0 mmol H_2/(l × h)	Moran and Shapiro, 1996
Thermophilic, undefined	198:0 mmol H_2/l/24 h	8:2 mmol H_2/(l × h)	Lindblad *et al*., 2002
Extreme thermophilic, pure strain[c]	8:4 mmol H_2/l/h	8:4 mmol H_2/(l × h)	Ko and Noike, 2002; Kondo *et al*.,2002

[a] *Clostridium species* #2.

[b] A consortium of unknown microorganisms cultured from a natural substrate and selected by the bioreactor culture conditions.

[c] *Caldicellulosiruptor saccharolyticus*.

2.8.2. Biohydrogen: Prospects for Practical Application

Our analyses indicate that photosynthesis-based systems do not produce H_2 at rates that are sufficient to meet the goal of providing enough H_2 to power even a 1 kW PEMFC on a continuous basis (Table 2.5). This does not mean that these systems should be abandoned.

Table 2.5: Is biohydrogen practical? The size of bioreactor required to power PEM fuel cells of different output

$BioH_2$ system	H_2 synthesis rate (mmol H_2 (l × h))[a]	Size of bioreactor required [b] to power a:			
		1.0 kW FC(l)	1.5 kW FC (l)	2.5 kW FC (l)	5.0 kW FC (l)
Direct photolysis	0.07	$3{:}41 \times 10^5$	$5{:}12 \times 10^5$	$8{:}56 \times 10^5$	$1{:}71 \times 10^6$
Indirect photolysis	0.355	$6{:}73 \times 10^4$	$1{:}01 \times 10^5$	$1{:}69 \times 10^5$	$3{:}37 \times 10^5$
Photo-fermentation	0.16	1.49×10^5	$2{:}24 \times 10^5$	$3{:}74 \times 10^5$	$7{:}58 \times 10^5$
CO-oxidation by R. gelatinosus	96.0	$2{:}49 \times 10^2$	$3{:}74 \times 10^2$	$6{:}24 \times 10^2$	$1{:}25 \times 10^3$
Dark-fermentations					
Mesophilic, pure strain[c]	21.0	$1{:}14 \times 10^3$	$1{:}71 \times 10^3$	$2{:}85 \times 10^3$	$5{:}70 \times 10^3$
Mesophilic, undefined[d]	64.5	$3{:}71 \times 10^2$	$5{:}57 \times 10^2$	$9{:}29 \times 10^2$	$1{:}86 \times 10^3$
Mesophilic, undefined	121.0	$1{:}98 \times 10^2$	$2{:}97 \times 10^2$	$4{:}95 \times 10^2$	$9{:}89 \times 10^2$
Thermophilic, undefined	8.2	$2{:}91 \times 10^3$	$4{:}38 \times 10^3$	$7{:}31 \times 10^3$	$1{:}46 \times 10^4$
Extreme thermophilic, undefined[e]	8.4	$2{:}85 \times 10^3$	$4{:}28 \times 10^3$	$7{:}13 \times 10^3$	$1{:}43 \times 10^4$

[a] Conrested units.

[b] Approximate volumes. Calculated volumes were rounded up to nearest whole value.

[c] *Clostridium species* #2.

[d] A consortium of unknown microorganisms cultured from a natural substrate and selected by the bioreactor culture conditions.

[e] *Caldicellulosiruptor saccharolyticus*.

Thermophilic and extreme thermophilic biohydrogen systems would require bioreactors in the range of approximately 2900–14; 600 l to provide sufficient H_2 to power PEMFCs of 1.5–5:0 kW, and a bioreactor of approximately 5700 l would be required to power the 5:0 kW fuel cell using the pure culture of mesophilic *Clostridium sp*. (Levin *et al*., 2004)

Some dark-fermentation systems and the CO – water shift reaction of R. *gelatinosus* CBS, however, appear promising. Chang et al reported bioreactors of reasonable size would be sufficient to power the 5.0 kW fuel cell using undefined consortia of mesophilic bacteria, enriched for *Clostridium* species. The system reported (Chang *et al*., 2002) in particular appears most promising. A bioreactor of approximately 500 l (495 l, in Table

2.4) would provide enough H_2 to power a 2:5 kW PEMFC, while a bioreactor of approximately 1000 l (989 l, Table 2.5) would provide sufficient H_2 to power a 5:0 kW PEMFC. The CO–water shift reaction of R. gelatinosus CBS Is intriguing as it offers the potential to capture and reform CO, and produce H_2. A bioreactor of approximately 624 l would be required provide enough H_2 to power the 2:5 kW PEMFC, while a bioreactor of approximately 1250 l (1247 l, Table 2.5) would provide sufficient H_2 to power a 5:0 kW PEMFC (Levin *et al*., 2004). With all this in place the commercialization of hydrogen and it's economy becomes very feasible and achievable

However, several reviews on MFC are available, each with a different flavor or emphasis. Logan *et al*. (2006) reviewed MFC designs, characterizations and performances. One of the primary applications of MFCs will likely be wastewater treatment, as a single process can be used to simultaneously accomplish both wastewater treatment and power generation (Feng *et al*., 2008, 2011; Liu *et al*., 2004; Min *et al*., 2005). MFCs are also being examined as biosensors (Di Lorenzo *et al*., 2009; Kim *et al*., 1999, 2003), and recently it has been shown that power densities can be increased through the incorporation of reverse electrodialysis stacks into the system (Kim and Logan, 2011). Through modification of MFCs, it is possible to accomplish additional goals, such as salt water desalination (Cao *et al*., 2009; Jacobson *et al*., 2011; Mehanna *et al*., 2010).

One of the challenges for scaling up MFCs and other bioelectrochemical systems is the development of compact reactors. So far, there have only been a few studies that describe larger scale reactors using multiple electrodes or chambers in MFCs (Dekker *et al*.,

2009; Zhang *et al*., 2010), or microbial electrolysis cells (Cusick *et al*., 2011; Rader and Logan, 2010).

The microbial metabolism in MFCs was reviewed by Rabaey and Verstraete (2005). Lovley (2006) mainly focused his review on the promising MFC systems known as Benthic Unattended Generators (BUGs) for powering remote-sensoring or monitoring devices from the angle of microbial physiologies. Pham *et al*. (2006) summarized the advantages and disadvantages of MFCs compared to the conventional anaerobic digestion technology for the production of biogas as renewable energy. Chang *et al*. (2006) discussed both the properties of electrochemically active bacteria used in mediatorless MFC and the rate limiting steps in electron transport. Bullen *et al*. (2006) compiled many experimental results on MFCs reported recently in their review on biofuel cells.

A real breakthrough was made when some microbes were found to transfer electrons directly to the anode (Kim *et al*., 1999a, Chaudhuri and Lovley, 2003). These microbes are operationally stable and yield a high Coulombic efficiency (Chaudhuri and Lovley, 2003; Scholz and Schroder, 2003). Shewanella putrefaciens (Kim *et al*., 2002), Geobacteraceae sulferreducens (Bond and Lovley, 2003), Geobacter metallireducens (Min *et al*., 2005a) and Rhodoferax ferrireducens (Chaudhuri and Lovley, 2003) are all bioelectrochemically active and can form a biofilm on the anode surface and transfer electrons directly by conductance through the membrane.

2.8.3. Microbes Used in Microbial Fuel Cells

Many microorganisms possess the ability to transfer the electrons derived from the metabolism of organic matters to the anode. A list of them is shown in Table 2.6 together with their substrates. Marine sediment, soil, wastewater, fresh water sediment and activated sludge are all rich sources for these microorganisms (Niessen *et al*., 2006, Zhang *et al*., 2006). A number of recent publications discussed the screening and identification of microbes and the construction of a chromosome library for microorganisms that are able to generate electricity from degrading organic matters (Logan *et al*., 2005; Holmes *et al*., 2004; Back *et al*., 2004).

Table 2.6: Microbes used in MFCs

Microbes	Substrate	Applications
Actinobacillus succinogenes	Glucose	Neutral red or thionin as electron mediator (Park and Zeikus, 2000; Park and Zeikus,1999; Park *et al*., 1999)
Aeromonas hydrophila	Acetate	Mediator-less MFC (Pham *et al*., 2003)
Alcaligenes faecalis, Enterococcus gallinarum, Pseudomonas aeruginosa	Glucose	Self-mediate consortia isolated from MFC with a maximal level of 4.31 W m−2. (Rabaey, 2004)
Clostridium beijerinckii	Starch, glucose, lactate, molasses	Fermentative bacterium (Niessen *et al*.,2004b)
Clostridium butyricum	Starch, glucose, lactate, molasses	Fermentative bacterium (Niessen *et al*., 2004b; Park *et al*., 2001)
Desulfovibrio desulfuricans	Sucrose	Sulphate/sulphide as mediator (Ieropoulos *et al*., 2005a; Park *et al*., 1997)
Erwinia dissolven	Glucose	Ferric chelate complex as mediators Vega and Fernandez, (1987)
Escherichia coli	Glucose sucrose	Mediators such as methylene blue needed. (Schroder *et al*., 2003; Ieropoulos *et al*., 2005a; Grzebyk and Pozniak, 2005)
Geobacter metallireducens	Acetate	Mediator-less MFC Min *et al*. (2005a)
Geobacter sulfurreducens	Acetate	Mediator-less MFC (Bond and Lovley, 2003; Bond *et al*., 2002)
Gluconobacter oxydans	Glucose	Mediator (HNQ, resazurin or thionine) needed Lee *et al*.,2002)
Klebsiella pneumonia	Glucose	HNQ as mediator biomineralized manganese as electron acceptor (Rhoads *et al*., 2005; Menicucci *et al*., 2006)
Lactobacillus plantarum	Glucose	Ferric chelate complex as mediators (Vega and Fernandez, 1987)
Proteus mirabilis	Glucose	Thionin as mediator (Choi *et al*., 2003; Thurston *et al*., 1985)
Pseudomonas aeruginosa	Glucose	Pyocyanin and phenazine-1-carboxamide as mediator (Rabaey *et al*., 2004, 2005a)
Rhodoferax ferrireducens	Glucose, xylose sucrose, maltose	Mediator-less MFC (Chaudhuri and Lovley, 2003; Liu *et al*., 2006)
Shewanella oneidensis	Lactate	Anthraquinone-2,6-disulfonate (AQDS) as mediator (Ringeisen *et al*., 2006)
Shewanella putrefaciens	Lactate, pyruvate, acetate, glucose	Mediator-less MFC (Kim *et al*., 1999a,b); but incorporating an electron mediator like Mn IV) or NR into the anode enhanced the electricity production (Park and Zeikus, 2002)
Streptococcus lactis	Glucose	Ferric chelate complex as mediators (Vega and Fernandez, 1987)

2.9. The Hydrogen Economy

The hydrogen economy is a proposed system of delivering energy using hydrogen. The term hydrogen economy was coined by John Bockris during a talk he gave in 1970 at General Motors (GM) Technical Center ("The History of Hydrogen" 2010).

The hydrogen economy has become an accepted term for an energy system innovation that strongly depends on hydrogen as key energy carrier. Many system studies of the hydrogen economy, however, deal almost exclusively with the technological, rather than

economic features of such a system. Additionally, a number of the studies that do focus on economic factors suggest that the hydrogen economy might not be economically feasible, not unless under favorable conditions are assumed for the hydrogen technology cost reductions (IEA, 2005; NRC, 2004; Barreto *et al*., 2003). This economic angle partially explains the position of hydrogen technologies in key global scenario studies for the medium term (up to 2030) such as the IEA World Energy Outlook (IEA, 2008b), where hydrogen is hardly mentioned.

2.9.1 The Basis for H_2 Economy

Population is a strong factor to the extinction of the world natural resources, an estimate increase of 35% in the world oil demand will occur over the next 30 years because of growth in the world's population (Nandi and Sengupta, 1998). Consequently 62% will be from population growth and rapid economic expansion from developing countries (Lattin and Utgikar, 2007). Hydrogen has the potential to lessen the worlds dependency on fossil fuels, but further research and technology is needed before a sustainable hydrogen economy can be established. Biological production of hydrogen by anaerobic fermentation is one such area of research which shows great potential produce hydrogen from biological methods that is less energy intensive than chemical or electrochemical methods because biological methods are normally carried out at ambient temperature and pressure (Jo *et al*., 2007).

Recent research regarding air pollution effects on human health describes serious lung damage sustained from fossil fuel combustion. Substituting hydrogen for fossil fuel will

result in improved physical health (Zweig, 1994). The main improvement with regard to the global warming problem can nonetheless only be achieved if renewable fuels are introduced (Wurster *et al*., 1998). More so, a major advantage of fuel cell vehicles (and all fuel cells) is that they represent an inherently clean, efficient and quiet technology and can optimize use of fuels from environmentally begin energy sources and feed stocks such as solar, wind, geothermal and biomass. One must emphasize these attributes to the maximum extent possible because there is increasing competition from conventional technologies (Lloyd *et al*., 1998).

Hydrogen production by fermentative bacteria is technically a simpler process over other biological processes because it proceeds at higher rates and does not require light sources (Han and Shin, 2003) and the hydrogen produced is clean. Despite being a clean and high energy fuel, currently only 50 million tons of hydrogen are traded every year with a growth rate of about 10% (Winter, 2005). The majority of this hydrogen is used to produce ammonia fertilizer, as feedstock for chemical and petroleum refining areas, plastics, solvents and other commodities (Dunn ,2002). Approximately 95% of hydrogen produced is consumed at the site of production with 1.5 million tons being sold for industrial and chemical uses (Lattin and Utgikar, 2007).

Anaerobic fermentation is also considered a simpler option because it allows the production of hydrogen by relatively straightforward procedures and can utilize substrates from many different sources (Nath and Das 2004). Several obstacles must be overcome before hydrogen from biological processes can be produced economically. In

the anaerobic process there are several stages that occur simultaneously. The last stage, methanogenesis, utilizes the intermediate products from the preceding stages and converts them into methane, carbon dioxide, and water (Parawira 2004).

2.9.2 Fundamental Factors Affecting the Implementation of the H_2 Economy

The hydrogen economy is coming with the impetus to transform our fossil energy-based society, which inevitably will cease to exist, into a renewable energy-based one (Ogden *et al*., 1999). However, this transformation will not occur overnight. However, President Bush, during his State of the Union Address in the year 2003, pronounced a $1.2 billion jump-start to the hydrogen economy. The move would represent not only freedom from U.S-dependence on foreign oil, which is a national security issue, but also a necessary and gargantuan step towards improving the environment by reducing the amount of carbon dioxide released into the atmosphere. However, hydrogen storage has proven to be one of the most important issues and potentially biggest blockade for the implementation of a hydrogen economy. Nonetheless, three options exist for storing hydrogen, in a solid, liquid, or gaseous state, the former is becoming accepted as the only method potentially able to meet the gravimetric and volumetric densities of the recently announced 'Freedom Car goals'; and of all known hydrogen storage materials, complex hydrides may be the only hope (Bush, 2003).

The major problem in utilization of hydrogen gas as a fuel is its unavailability in nature and the need for inexpensive production methods (Kapdan and Kargi, 2006). Hydrogen production costs from natural gas using steam methane reforming (SMR) from about 1.50 US$/kg at large-scale facilities (1.2 Gg/d) to about 3.75 US$/kg at a 500 kg/d facility

(assumes 7 US$/GJ natural gas price) (Williams *et al*., 2007). Hydrogen production by gasification and pyrolysis of biomass are not generally considered economically competitive with SMR processes. The price of hydrogen obtained by direct gasification of lignocellulosic biomass, however, is about three times higher than that for hydrogen produced by SMR (Spath *et al*., 2003). According to Hamelinck and Faaij (Hamelinck and Faaij, 2002), the cost of producing hydrogen from biomass ranges from 10 to 14 US$/GJ, with a net higher heating value (HHV) energy efficiency of 56-64%. It is believed that in the future biomass can become an important sustainable source of hydrogen. The future prospects for hydrogen economy or economic hydrogen production are the basic point of many articles (Veziroglu, 1998). Several studies have shown that the cost of producing hydrogen from biomass is strongly dependent on the cost of the feedstock (Balat and Balat, 2009). Hydrogen from biomass gasification is not expected to develop in the near term due to costs, lack of demonstrated technology and lack of widespread hydrogen market and infrastructure (Williams *et al*., 2007). The maturity and realization of the hydrogen economy is cost dependent (i.e. cost is a critical factor). In accordance with the above, the cost of electrolytic hydrogen is comparable to synthetic hydrocarbon fuels, and about three times as expensive as hydrogen from fossil fuel sources (Figure 2.4) (Peschka, 1996)

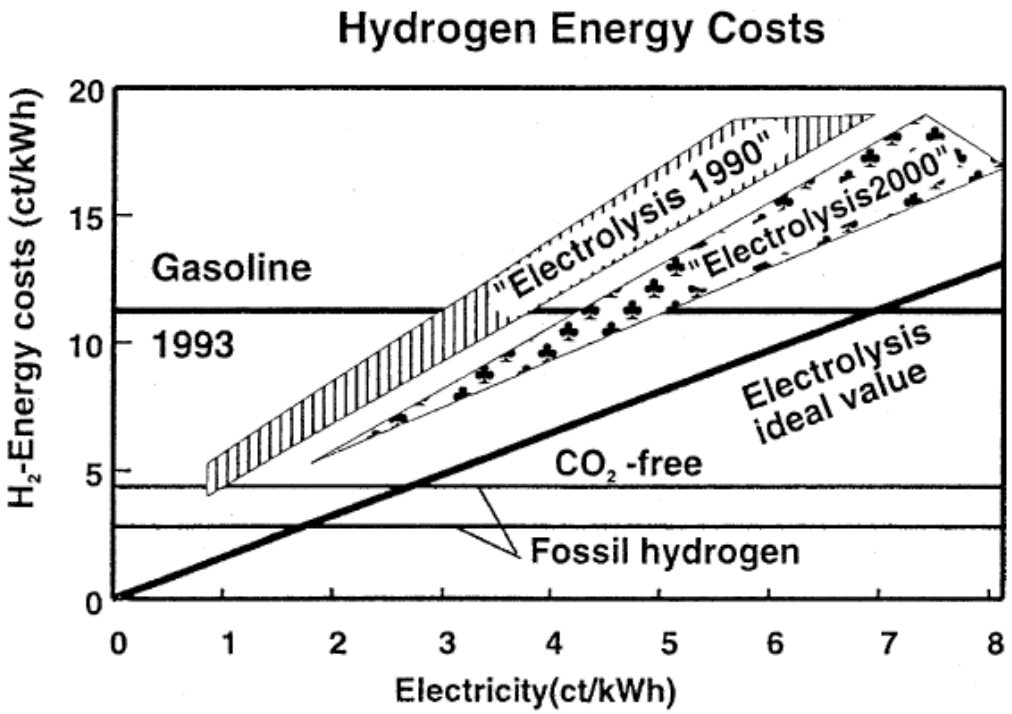


Figure 2.4: Comparison of hydrogen costs.

Nonetheless, figure 2.5 shows a breakdown of the budget for the DOE's Hydrogen Program, by agencies within the DOE and among research sectors of the office of Energy Efficiency and Renewable Energy (EERE). Eighteen percent of the 2008EERE hydrogen budget was directed at developing hydrogen production and delivery pathways, and only 11% of that budget was invested in researching biological production pathways. Given this level of investment by the only program aimed at launching the U.S. hydrogen economy, the hurdle is set high for establishing biohydrogen as a feasible and competitive sustainable technology.

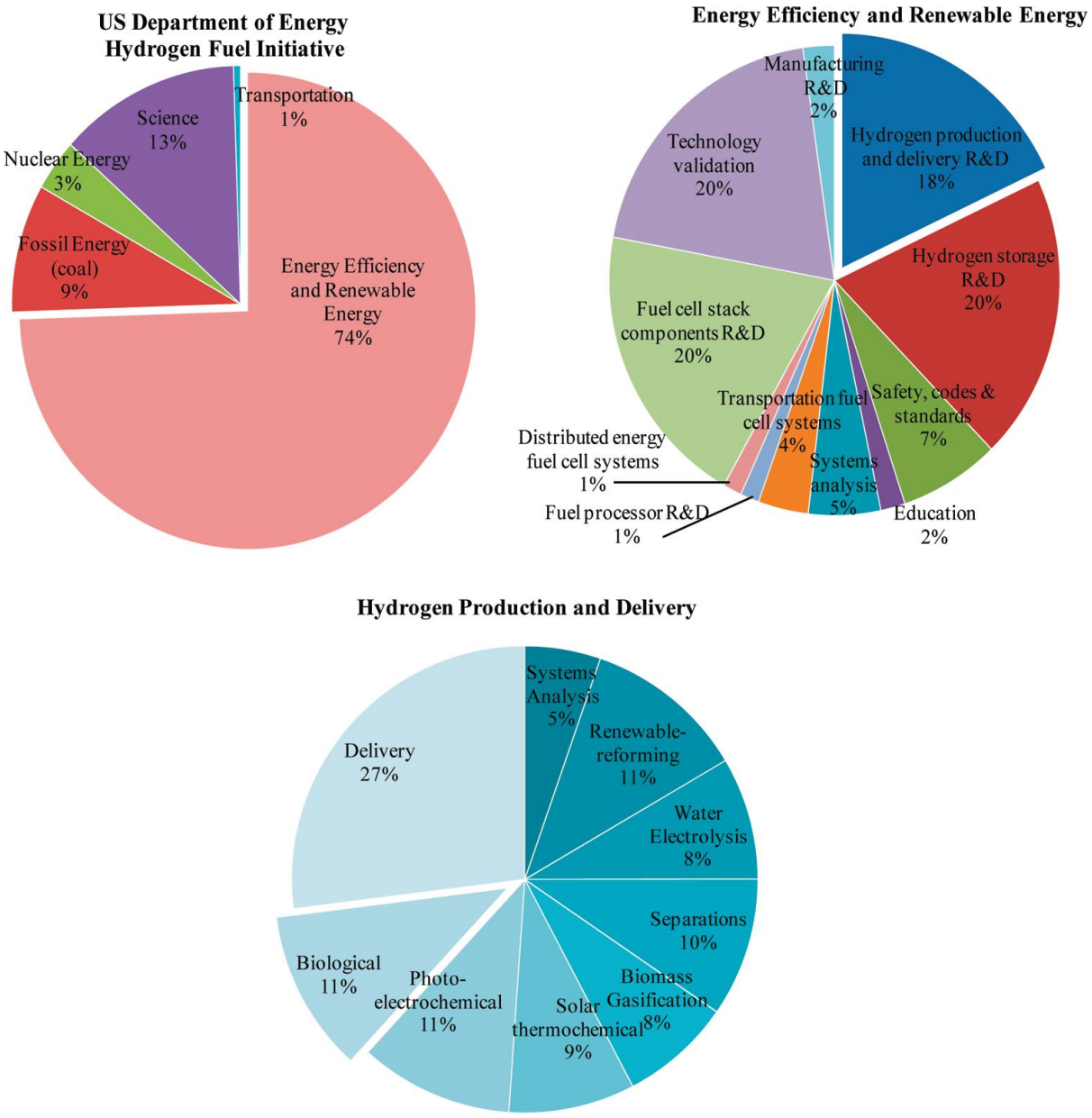


Figure 2.5: Budget breakdown for the Hydrogen Fuel Initiative for the U.S. DOE, Energy Efficiency and Renewable Energy (EERE) sector, and Hydrogen Production program within the EERE for the fiscal year 2008. The total Hydrogen Fuel Initiative budget for the U.S. DOE for 2008 was $281 million.

In conclusion, the current biohydrogen technology is not ready for industrial scale production of hydrogen. A decentralized strategy may be more appropriate for hydrogen to reduce costs associated with transport and storage of the elusive, small molecule sized gas. A major recommendation for the U.S. DOE Hydrogen Initiative from the National Academy of Engineering is to explore decentralized systems for hydrogen energy (National Research Council and National Academy of Engineering 9780309091633). Applications could include on-site production for hydrogen fueling stations for fuel-cell vehicles or on site fermentors with direct links to fuel cells for home-based energy. Levin et al. (Levin *et al*., 2004) calculated that a bioreactor of approximately 1000 L, with a hydrogen production rate of approximately 2.95 L L^{-1} h^{-1}, connected to a proton exchange membrane fuel cell (PEMFC) of 5.0 KW (50% efficiency, 95% utilization) could be used to provide sufficient energy to meet an average residential electricity load. The next phase of biohydrogen research needs to include pilot scale demonstration projects to explore the opportunities for industrial scale production, as described by James et al. (James *et al*., 2009), as well as localized production and energy generation.

Hydrogen safety research was initiated decades ago as a result of accidents in the process industries. Initially it was supported by safety research for nuclear power plants and aerospace industries. In recent times it has been supported in Europe and countries of the International Partnership for the Hydrogen Economy (IPHE) worldwide in order to provide safety of hydrogen as an energy carrier for the emerging hydrogen economy. Hydrogen is used as an energy carrier for various fuel cell technologies, alternative fuel

vehicles, combined heat and power units for stationary application, etc. (Molkov *et al*., 2006).

2.9.3 Role of Hydrogen in the Future Global Energy Supply

Energy demand has grown strongly and will continue to increase, predominantly in developing countries where energy is desired for economic growth and poverty alleviation. However, the rational use of energy becomes a keyword for the world sustainable development both in developed and developing countries (Marechal *et al*., 2005). Projected world primary energy demand by 2050 is expected to be in the range of 600-1000 EJ (compared to about 500 EJ in 2008) (IEA, 2009). At the present time primary energy sources are dominated by fossil fuels, with nearly 80% of global energy demand supplied from crude oil, natural gas, and coal (Evans, 2007). Petroleum-based fuels are limited reserves concentrated in certain regions of the world. These sources are on the verge of reaching their peak production. Known petroleum reserves are estimated to be depleted in less than 50 years at the present rate of consumption (Sheehan *et al*., 1998). As supplies of fossil fuels dwindle and concerns about continued contributions of additional carbon dioxide to the atmosphere intensify, there is an increasing need for new sources of energy from renewable carbon-neutral sources with minimal negative environmental impact (Lovley, 2006). Renewable energy is projected to play a key function in the global future energy provision. Hydrogen and fuel cells are often considered as a key technology for future sustainable energy supply. Renewable shares of 36% (2025) and 69% (2050) on the total energy

Table 2.7: Share of individual primary energy sources in meeting final energy needs (%)

Source of Energy	1998	2025	2050
Fossil Fuel	88	62	29
Nuclear energy	10	2	2
Hydrogen from solar energy	-	7	31
Electricity from solar energy	-	11	16
Heat from solar energy	-	18	22
Energy from solar energy	2	25	35
Hydrogen	-	11	34

(Rohland *et al*., 1992)

demand will lead to hydrogen shares of 11% in 2025 and 34% in 2050 (Rohland *et al*., 1992). The share of individual primary energy sources in meeting final energy needs are given in Table 2.7. Carbon dioxide is a major greenhouse gas associated with global warming, and is produced in all combustion processes involving fossil fuels as well in other industrial processes such as cement production and sweetening of natural gas (Keskin and Emiroglu, 2010). One-fifth of global carbon dioxide emissions are created by the transport sector, which accounts for some 60% of global oil consumption (Balat and Balat, 2009). Consequent upon this, an alternate transportation fuels, such as ethanol, biodiesel, and hydrogen, will play an important role in the world's future.

Due to the increasing mobility of people and goods, the transport sector accounts for more than 30% of final energy consumption in the European Union (EU) and is expanding (Malc and Freire, 2006). The European Commission White Paper (European transport policy, 2010) calls for dependence on oil in the transport sector to be reduced by

using alternative fuels such as biofuels. An increasing use of biofuels for transport is emerging as an important policy strategy to substitute petroleum-based fuels (Malc and Freire, 2006). As is evidenced by several funded programs from many national government agencies all over the world, hydrogen is being promoted as the fuel for the future (Saxena, 2003). Figure 2.6 shows the shares of alternative fuels compared to the total automotive fuel consumption in the world as a futuristic view .(Demirbas, 2009)

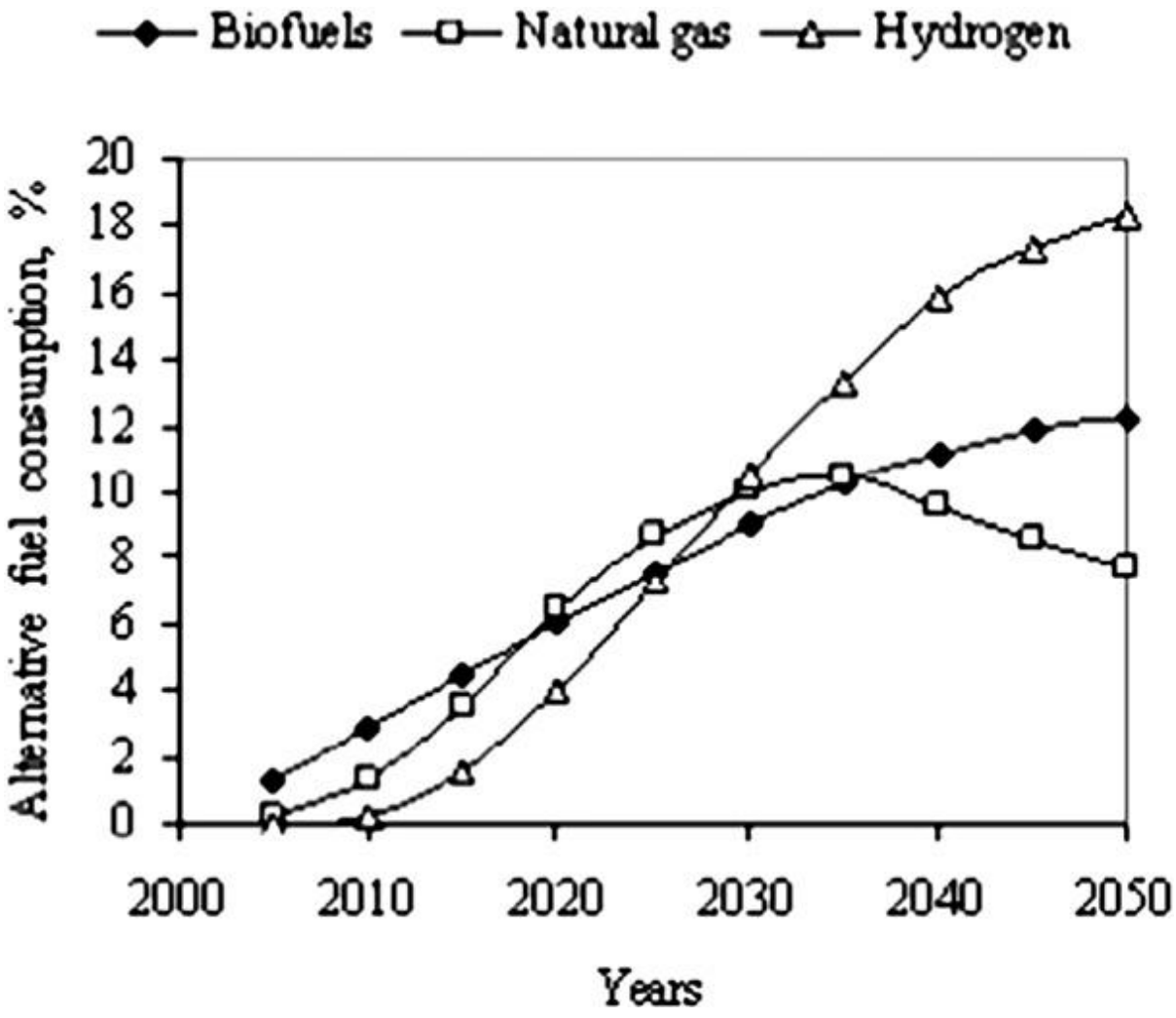


Figure. 2.6: Shares of alternative fuels compared to the total automotive fuel consumption in the world.(Demirbas, 2009).

2.10. Current Trend in Biofuel State of the Art

So far, no experience with commercial production of second-generation biofuels yet exists. In particular, in developing countries it will be a challenge to balance large-scale industrial development with small-scale local value chains, which would be required to ensure environmental, economical and social sustainability (OECD/IEA, 2010)

Expert assessments in the reviewed studies varied greatly, from 33 EJ/yr in 2050 (Hoogwijk *et al.,* 2003) assuming that mainly agricultural and forestry residues are available for bioenergy production. In the most ambitious scenario (Smeets *et al.,* 2007), the bioenergy potential reaches roughly 1 500 EJ/yr in 2050. The scenario assumes availability of 72% of current agricultural land for biofuel production, mainly through increased yields and more intensive animal farming.

The *World Energy Outlook 2009* (IEA, 2009a) *450 Scenario1* projects biofuels to provide 9% (11.7 EJ) of the total transport fuel demand (126 EJ) in 2030. In the *Blue Map Scenario*2 of *Energy Technology Perspectives 2008* (IEA, 2008b) that extends analysis until 2050, biofuels provide 26% (29 EJ) of total transportation fuel (112 EJ) in 2050, with second-generation biofuels accounting for roughly 90% of all biofuel. More than half of the second-generation biofuel production in the *Blue Map Scenario* is projected to occur in non-OECD countries, with China and India accounting for 19% of the total production.

Ambitious biofuel support policies have recently been adopted in both the United States (with 60 billion litres of second-generation biofuel by 2022) and the European Union (with 10% renewable energy in the transport sector by 2020). Due to the size of the two

markets and their considerable biofuel imports, the US and EU mandates could become an important driver for the global development of second-generation biofuels, since current IEA analysis sees a shortfall in domestic production in both the US and EU that would need to be met with imports (IEA, 2009b).

Comparably low feedstock prices, in the range of USD 1-8/GJ, were indicated for Brazil, China, India, Mexico, South Africa and Thailand. Using the latest IEA production cost analysis, theoretical production costs for second-generation biofuels from straw or stalks are currently in the range of USD 0.60-0.79/lge in South Africa and up to USD 0.86/lge in India and China (Table 2.8). This is still high compared to the reference gasoline price of USD 0.43/lge (*i.e.* oil at USD 60/bbl), but in the long term, technology improvement, higher conversion efficiencies and better transport logistics could bring costs close to the gasoline reference, if costs for feedstocks would remain stable.

Table 2.8: Theoretical production price for second-generation biofuels in selected countries

Oil Price: USD 60/bbl		Feedstock Price*	USD/lge	
			Btl-diesel	lc-Ethanol
Woody Energy Crops	Global (IEA analysis)	5.4	0.84	0.91
Straw/stalks	China	1.9 - 3.7	0.66 - 0.79	0.68 - 0.85
	India	1.2 - 4.3	0.62 - 0.80	0.63 - 0.86
	Mexico	3.1	0.74	0.79
	South Africa	0.8 - 3.1	0.74 0.6	0.6 - 0.79
	Thailand	2.0 - 2.8	0.67 - 0.72	0.67 - 0.77

*Note that feedstock prices reflect assumptions by local experts and might vary regionally. Assumed cost factors are: capital costs: 50% of the total production costs; feedstock is 35%; operation and maintenance (O&M), energy supply for the plant and others between 1-4% each.
Source: Based on IEA analysis presented in ***Transport, Energy and CO_2*** (IEA, 2009c)

Presently many experts still think that hydrogen has a major role to play as an energy carrier in future energy supply (Veziroglu, 2008; Balat, 2008; Eggertson, 2004) But however, it's process methodology is currently more expensive than conventional energy sources. In the longer-term renewables will become the most important source for the production of hydrogen. Hydrogen will play an important role in a future energy economy mainly as a storage and transportation medium for renewable energy sources. Renewable shares of 69% on the total energy demand will lead to hydrogen shares of 34% in 2050 (Table 2.9) (Demirbas, 2009)

Table 2.9: Share of individual primary energy sources in meeting final energy needs (%).
Source: Ref.(Demirbas, 2009)

Source of energy	1998	2025	2050
Fossil fuels	88	62	29
Nuclear energy	10	2	2
Hydrogen from solar energy	–	7	31
Electricity from solar energy	–	11	16
Heat from solar energy	–	18	22
Energy from solar energy	2	25	35
Hydrogen	–	11	34

Hydrogen produced from biorenewables is a sustainable energy carrier for promising alternative to fossil fuels. Advantages of biomass-based hydrogen includes energy security reasons, environmental concerns, foreign exchange savings, and socioeconomic issues related to the rural sectors of all countries in the world. Due to its environmental merits, the share of hydrogen from biomass in the automotive fuel market will grow fast in the next decade (Elif Kırtay, 2011)

CHAPTER THREE

THE INFLUENCE OF BACTERIAL BIOMASS DENSITY ON HYDROGEN PRODUCTION EFFICIENCY IN A THERMOPHILIC GRANULAR BED BIOREACTOR

3.1. Introduction

Recently significant progress has been made in the development of anaerobic fluidized bacterial granular bed bioreactors (*AFGB*) that have high volumetric hydrogen productivities (Lee *et al*., 2004; Lee *et al*., 2006; Thompson *et al*., 2008; O-Thong *et al*., 2008; Zhang *et al*., 2007a, 2007b, 2008b, 2008c; Ngoma *et al*., 2011). The high volumetric hydrogen productivities (*HPs*) has been made possible through the development of a number of different experimental procedures that facilitate the rapid initiation, growth and development of bacteria granules (Lee *et al*., 2004; Thompson et al., 2008; O-Thong *et al*., 2008; Zhang *et al*., 2007/8; Ngoma *et al*., 2011). Because bacterial granules have good settling properties high volumetric biomass densities can be maintained even under high dilution rates without significant biomass loss through washout. This feature has been major a advantage of *AFGB* systems. While high biomass densities obviously result in high volumetric hydrogen productivities (*HP*) it is not clear how biomass densities influence hydrogen production efficiencies. Hydrogen production efficiencies can be expressed in terms of mol of H_2 produced per mol of glucose (*HY*) and in terms of mol of H_2 produced per g of bacterial biomass per h (*SHP*).

The objective of this study was to induce granule formation from a mixed bacterial cultured adapted to grow at a thermophilic temperature of 70^{o}C, and then to

investigate how various biohydrogen production parameters such as the *HPs*, *HYs*, % H_2 and specific hydrogen productivity *SHP* were influenced by bacterial biomass densities, and how this influence can be modulated by effluent recycling rates.

3.2. Materials and Methods

3.2.1. Medium

An Endo formulation (Endo *et al*., 1982; Thompson *et al*., 2006) was used as the nutrient medium for inoculum preparation and for the bioreactor experiments. The medium contained 17.8 g sucrose/L and the following mineral salts (g. L^{-1}): NH_4HCO_3 6.72, $CaCl_2$ 0.2, K_2HPO_4 0.699, $NaHCO_3$ 3.36, $MgCl_2.6H_2O$ 0.015, $FeSO_4.7H_2O$ 0.0225, $CuSO_4.5H_2O$ 0.005, and $CoCl_2.H_2O$ 1.24 x 10^{-4}g.

3.2.2. Inoculum preparation

An undefined extreme thermophilic anaerobic bacterial consortium was derived from a mixture of sewage sludge and fresh cow dung. Sewage sludge was obtained from the overflow outlet of a mesophilic anaerobic digester at the Olifantsvlei wastewater treatment works (Johannesburg). Fresh cow dung was obtained from grass fed dairy cows at the Animal and Dairy Research Institute (Irene), Gauteng. Sewage and dung samples were incubated in Endo medium (50% v/v) at 90 ºC for 2 hours. After the heat treatment the pH of the samples were reduced to pH 2.0 with 0.1 N HCl. Inoculum samples were kept at this pH in sealed airtight Schott bottles for 12 h at room temperature and then readjusted to pH 7.0 by mixing with Endo medium (50% v/v). The two inoculum

preparations, sewage (1 L) and dung (1 L) were then applied immediately to the bioreactor.

3.2.3. Bioreactor design and set-up

The bioreactor system of Ngoma *et al* (2011) was modified to facilitate the stable maintenance of high biomass densities within the working volume of the bioreactor. The bioreactor consisted of 4 components: an influent and recycled effluent inlet manifold or diffuser, tubular bioreactor, a liquid-solid separator or sedimentation column connected to the top end of the tubular bioreactor and a tubular gas-disengager (Figure 3.1). Clear Perspex hollow tube was used for the construction of the tubular bioreactor (internal diameter (ID): 80 mm; height (H): 1000). The working volume for the tubular bioreactor's fluidized bacterial granular bed was 5 L. A 11.6 L liquid-solid separator was connected to the top end of the tubular bioreactor for solid-liquid separation to prevent the washout of the granules from bioreactor, especially when the bioreactor was operated at high influent rates and high effluent recycle rates during the night period. The solid - liquid separator consisted of two parts: a 5.3 L component (ID: 150 mm and H: 300 mm), and a 6.3 L component (ID: 200 mm and H: 200 mm). At the base of the bioreactor the clear Perspex cylinder was connected to a conical shaped diffuser (ID: 80 mm and H: 150 mm) made from PVC which functioned as the primary inlet for the effluent recycle stream. A stainless steel sieve (32 mesh) was fixed over the inlet of the diffuser. Above the stainless steel sieve the conical diffuse was filled with a 100 mm layer of 5 mm glass beads. Positioned at the upper end of the diffuser were 4 inlet ports (ID 5 mm) with each inlet arranged at 90^{o} with respect to the two other inlets on each side. Nutrient medium

(influent stream) was supplied directly into the upper glass bead layer via the 4 inlet ports. The effluent overflow from solid-liquid separator was decanted into a gas-disengager which consisted of a gas collection cylinder (H: 200 mm and ID: 150 mm) connected to a gas-disengager cylinder (H: 600 mm and ID: 60 mm). The gas-disengager had two effluent outlets, one at the bottom that was connected to a variable Boyser® Bonfiglioli AMP-16 peristaltic pump (0.37 kW) which was used to recycle de-gassed effluent into the bioreactor via the diffuser. For effluent recycling the pump was set at values between 15 rpm and 50 rpm which gave a variable volumetric pumping rate ranging from 1.3 L/min to 3.5 L/min, with latter value representing the maximum possible pumping rate of the pump. The second effluent outlet drained the excess effluent overflow from the gas-disengager. The gas-disengager gas-outlet port was connected to a gas meter (Ritter drum-type gas meter TG 05/3). All Ritter drum gas meter measurements were carried out 25^{o}C. The liquid-gas separator or gas-disengager had a working volume of 1.54 L and the total fluid occupied volume of the interconnecting piping was 1.9 L. Total fluid containing volume of the bioreactor system (bioreactor bed, solid-liquid separator, gas-disengager, diffuser, and piping) was 20.0 L. Bioreactor and gas-disengager temperatures were maintained at the two operational temperatures, 60 ºC and 70 ºC, by circulating heated water from a heated water bath through the bioreactor and gas-disengager water jackets. A Watson-Mallow (model 520U) peristaltic pump (Falmouth, UK) was used to pump the Endo nutrient into the bioreactor.

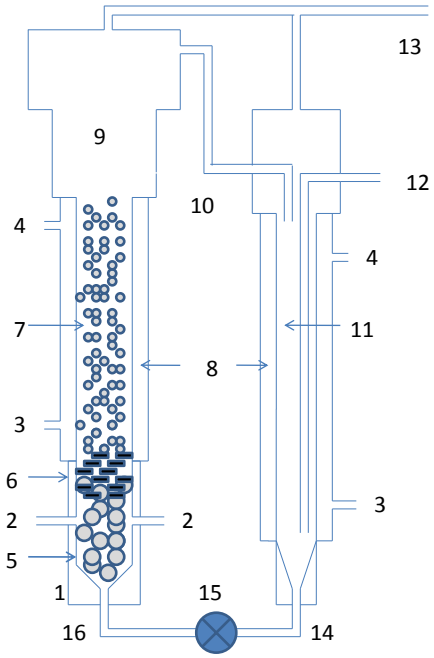


Figure 3.1: *AFGB* system. diagram labels: 1 – inlet manifold or diffuser; 2 – influent inlets; 3 – water jacket inlet for heat exchanger; 4 – water jacket outlet for heat exchanger; 5 – bed of glass beads in effluent/influent diffusion and cavitation for bubble generation; 6 – activated carbon for inducing granulation; 7 – fluidized bacterial granular bed (*B*); 8 – water jacket for heater exchanger; 9 – solid-liquid separator column or sedimentation column (*S*) for decanting effluent decanter; 10 – effluent connecting pipe to gas disengage (*P*); 11 – effluent gas disengager tube (*G*); 12 – effluent outlet overflow pipe (source of waste heat for heat recycling); 13 – gas outflow pipe (source of waste heat for heat recycling via heat-pump) ; 14 - effluent recycle outlet pipe (*P*); 15 – effluent recycle pump; 16 – effluent recycle inlet (*P*). Total *AFGB* volume: $V = B + S + G + P$.

3.2.4. Effluent recycle rate and effluent gas disengagement

Apart from bacterial biomass density the other fundamental bioreactor operation factor was the rate degassed effluent recycling through the expanded granular bed and into the gas disengager. Actual dissolved H_2 concentrations in dark anaerobic bioreactor can be between 30 and 80 fold higher than the predicted theoretical thermodynamic equilibrium values derived from the head space H_2 partial pressures using Henry's law (Pauss *et al*., 1990) Given the high rates of H_2 gas generation by anaerobic fluidized granular bed bioreactors and the low solubility of H_2, the H_2 contained in the effluent would be partitioned into two components: solubilized H_2 and non-solubilized H_2. Non-solubilized H_2 would consist of H_2 molecules trapped in the liquid phase in the form of microscopic bubbles or as aggregated clumps of H_2 molecules trapped within a matrix of H_2O molecules. Non-solubilized H_2 would be undergoing rapid dynamic reversible exchanges with solubilized H_2 resulting in a super-saturated equilibrium concentration of soluble H_2 in the liquid phase within the bioreactor. Under steady-state conditions the difference between the actual total concentration H_2 entrapped in the effluent relative to the predicted dissolved thermodynamic equilibrium concentration can be estimated from equation 3.1 (Kuroda *et al*., 1991).

$$\frac{H_2^L}{H_2^{L^*}} = \frac{HP_L}{K_H^T RTk_L} + 1 \qquad (3.1).$$

Where, $H_2{}^L$ (mol/L) is the supersaturated concentration of dissolved hydrogen in the bioreactor liquid phase, $H_2{}^{L^*}$ (mol/L) is the thermodynamic equilibrium dissolved hydrogen concentration, HP_L (mol/(L.h)) is the volumetric hydrogen productivity, $K_H{}^T$

(mol/ Pa) is Henry's constant, k_L (mol/(L.h)) is the H_2 volumetric mass transfer coefficient, R (8.314 m^3 Pa/(mol.K)) is the ideal gas constant, and T (K) is temperature.

Under ideal conditions the thermodynamic equilibrium concentration of dissolved hydrogen is related to partial pressure by Henry's law (equation 3.2) as follows

$$p = K_H^{T^o} H_2^{L^*} \qquad (3.2).$$

where p is the partial pressure of hydrogen, $K_H{}^T$ equals 1282.05 L.atm/mol at T^o = 298 K. The function of the effluent gas disengager was to reduce to the total concentration of H_2 trapped in the effluent to its thermodynamic equilibrium concentration. This was accomplished by facilitating the maximum transfer or release of H_2 from the liquid phase within the gas disengager to the vapour phase, which in turn was being continuously exhausted from the gas disengager.

Effluent discharge force into the gas disengager was dependent on the effluent recycle rate. High rates of effluent recycling between the bioreactor and the gas disengager generated a high degree of fluid turbulence and cavitation within the gas disengager tube. This vigorous mixing process within the gas disengager facilitated the release of undissolved H_2 from the effluent through bubble production. Efficient removal of undissolved or non-solubilized H_2 trapped in the effluent phase by gas disengagement was expected to increase the overall biohydrogen production efficiency of the bioreactor system.

3.2.5. Bacterial granule induction

On top of the glass bead bed a 100 mm bed of cylindrical activated carbon (*CAC*) particles (diameter = 2.5 mm and length = 5.0 mm) was used to facilitate the induction of bacterial granulation in the bioreactor under thermophilic conditions (Figure 3.2) (1). Prior to its use, the *CAC* was first washed with distilled water to remove all the suspended fine particles and then sterilized by autoclaving for 20 minutes. Concentrated (3x) Endo medium (18.0 L) and seed inoculum (2.0 L) was added to the bioreactor system. Following inoculation the bioreactor was operated on a batch effluent-recycle mode for 48 h at 70^{o}C to acclimatize the bacteria to thermophilic conditions and to allow for their attachment to the *CAC*. After this acclimatization period the bioreactor operation was then switched to continuous – effluent recycle mode with an initial hydraulic retention time (*HRT*) of 8 h, supplying Endo medium at its normal concentration. The *HRT* was then gradually decreased over 2 day intervals by increasing the nutrient medium supply rate. As the *HRT* was decreased from 8 to 4 h the growth and development of bacterial biofilm on the *CAC* particles became visible. With further decreases in the *HRT* below 4 h the biofilm growth increased and bacterial granules began to form and accumulate at the surface of the expanded *CAC* bed. Once granule formation had been initiated, further reductions in the *HRT* to between 2 and 1.6 h resulted in the rapid growth and expansion of the granular bed. Granule induction, initial growth and initial development was carried out at 70^{o}C.



Figure 3.2: Different stages of *AFGB* system (A) *AFGB* column with activated carbon carrier (*CAC*) (B) *CAC* coated with bacterial flocs (biofilms) after granulation has been induced. (C) Expanded bed as a result of high organic loading rate, effluent degassed recycle rate and reduced *HRT*. (D) Settled bed

3.2.6. Bacteria biomass

For estimation of bacterial granule biomass density, 20 ml of a settled suspension of granules were collected by filtration onto pre- weighed Whatman No1 90 mm filter disks and then dried at 70 ^{o}C for 48 h.

3.2.7. Granule settling velocity

Granules where placed at the top of a settling column (length: 1000 mm, diameter 80 mm) filled with Endo medium. Average settling velocity was calculated from the time taken for the granules to settle on the bottom of the column.

3.2.8. Granule diameter

Gross morphology of granules were assessed using the dissecting microscope. Granule diameters were estimated using a dissecting microscope (Olympus SZ-CTV) and image analysis was done with ScopeTek Scopephoto micro-image analysis software.

3.2.9. Features of granule bacteria

The visualization and interpretation of bacteria features were made possible by using JEOL JSM 840 scanning electron microscopy (SEM) equipped with a digital Nikon F301 camera. However, this is used when certain bacteria features cannot be shown with a light dissecting microscope for proper characterization of bacterial gross morphology. The microscope was operated at 20 KV and the microbial compositions were studied by standard gram stain technique.

3.2.10. Analytical techniques

Gas chromatography was used to analyze % gas composition (H_2, CO_2 and CH_4). A Clarus 500 GC PerkinElmer equipped with a thermal conductivity detector was used. The temperatures of injector, detector and column (PerkinElmer Elite Q Plot capillary column 30 m x 32 mm) were kept at 250 °C, 200 °C and 45°C , respectively. Argon was used as

the carrier gas at a flow rate of 2.0 ml min^{-1}. Sample gas injection volume was 40µl. The following formula (equation 3.3) was used for converting total bioreactor gas flux (L/h) to mmol H_2/h,

$$\frac{\Delta H_2}{\Delta t} = \frac{P\left[\left(\%H^{GC}\right)\frac{\Delta V}{\Delta t}\right]}{RT} \qquad (3.3).$$

Where, $\Delta H_2/\Delta t$ = mmol H_2 /h; P = atmospheric pressure (85 kPa); ($\%H_2{}^{GC}$) = percentage hydrogen content from GC measurements; $\Delta V/\Delta t$ = L/h of total gas production from the gas meter measurements; R is the gas constant (8.314 J/K/mol); T = 298.15 K (the temperature at which the gas flow from the bioreactors were monitored).

The concentration of sucrose in the bioreactor influent and effluent streams was determined using the sucrose-resorcinol method (Kerr *et al*., 1984)

3.2.11. Units used for hydrogen production and yield

A number of different units have been used hydrogen production. Total hydrogen production by the bioreactor has been expressed in the following units L H_2/h and mol H_2/h. Volumetric hydrogen productivity (*HP*) has been expressed in the following units L H_2/L/h and mmol H_2/L/h. Specific hydrogen productivity (*SHP*) represents H_2 production per g bacterial dry mass per h has been expressed in the following units L H_2/g/h and mmol H_2/g/h. While the substrate used was sucrose, hydrogen yield (*HY*) has been expressed in terms of glucose in the following units mol H_2/mol glucose.

3.2.12. Experimental design

After granule formation was initiated the influent rates were increased step-wise as follows: 5.4 L/h, 6.3 L/h, 7.2 L/h, 8.1 L/h, 9.0 L/h, 9.9 L/h, 10.8 L/h, 11.7 L/h, 12.6 L/h and 13.5 L/h. The bioreactor was operated in such a fashion for 50 days. Effluent recycle rate was maintained at 3.5 L/min. The effect of increasing effluent recycle rates (1.3 L/min, 1.6 L/min, 2.0 L/min, 2.3 L/min, 2.6 L/min, 2.9 L/min, 3.2 L/min, 3.5 L/min) on *HP*, *HY*, and 3. *SHP* was also determined.

In this study the main experimental focus was try and get some understanding on what factors influence the simultaneous maximization of *HP* and *HY*. Therefore the entire experimental strategy focused entirely of measuring total sucrose consumption and total hydrogen production so that values for *HP* and *HY* could be generated. Because these experiments were large experiments with huge numbers of measurement the scope of quantitative studies was narrowed down to colorimetric assays of sucrose and GC measurement of H_2 and any addition variables such as volatile fatty acids was excluded. Carbon dioxide production was monitored in all gas measurements and CO_2 was dependent on % H_2 content, and ranged from 30 – 40 % . Experiments were conducted and repeated with the same bioreactor a minimum of three times.

3.3. Results

3.3.1. Granule growth/dynamics and bioreactor operation stability

Following the inoculation of the bioreactors granule formation took place within 5 days after the Endo supply rate or influent rate had reached 5.4 L/h. There was full decoupling

of the suspended solids retention times and hydraulic retention was achieve at all effluent recycles rates. All suspended solid were present in the form of bacteria granules, however only planktonic bacteria were lost. At the end of 50 days the settled bed height of the granule bed had grown to 47 cm corresponding to a total bacterial dry mass of 141.8 g, giving a maximum biomass density of 28.4 g/L. The 11.6 L liquid-solid separator prevented the loss of granules through gas sludge piston induced washout at all supply rates and effluent recycle rates. The mean granule diameter (Figure 3.3) was 3.1 mm (SD = 0.36) and the mean granule settling velocity was 3.5 cm/s (SD = 0.29).



Figure 3.3: The different stages in bacterial granule growth using light dissecting microscope with its mean diameter estimated. Compact spherical granules were mainly observed in thermophilic *AFGB*. (A and B) depicts the lag phase bacterial growth. (C and D) shows matured granules over a long period after granulation.

A careful analysis of the bioreactor sample by gram staining shown that *AFGB* bacterial population comprises of a singular and binary rod- shaped bacteria (Figure 3.4). Nonetheless, comprehensive morphological studies of granules were established with the use of a scanning electron microscope (Figure 3.5). Sample under SEM examination revealed that microbial morphology comprised of long rod-like shaped bacteria predominated on the surface of the biofilm and granules, similar to acidogenic H_2-producing bacteria *Clostridium* sp. (Chen *et al*., 2005: Lee *et al*., 2006; Zhang *et al*., 2008b). It was also observed that, on the surface of bacterial granules there exists a porous inner structure or presence of cavities. Such structure is likely to facilitate the passage of nutrients and substrate as well as the release of metabolic products such as release of biogas (H_2) from the granules.

The bacteria were strongly attached to each other by extracellular polymeric substances (EPSs). The EPS play an important role in the promotion of the initial attachment of cells to solid surfaces; formation and maintenance of micro-colony and mature biofilm structure; and enhanced biofilm resistance to environmental stress and disinfectants. In some cases, EPS matrix also enables the bacteria to capture nutrients (Czaczyk and Myszka, 2007). The production of EPSs by some microorganisms under certain conditions is considered as the factor responsible for the phenomenon of anaerobic granulation (Dolfing, 1987).

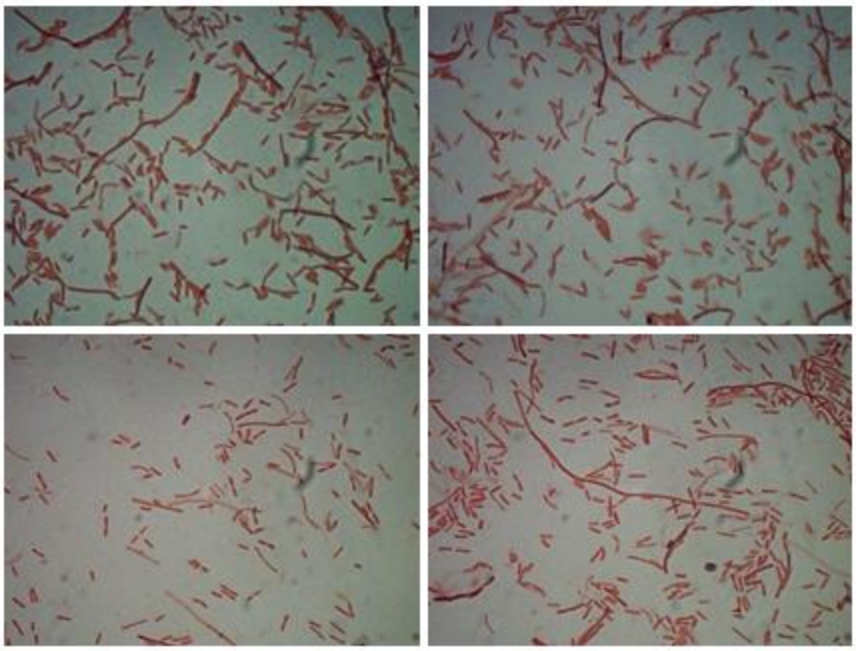


Figure 3.4: Shows spore-forming rod-like shaped cells.

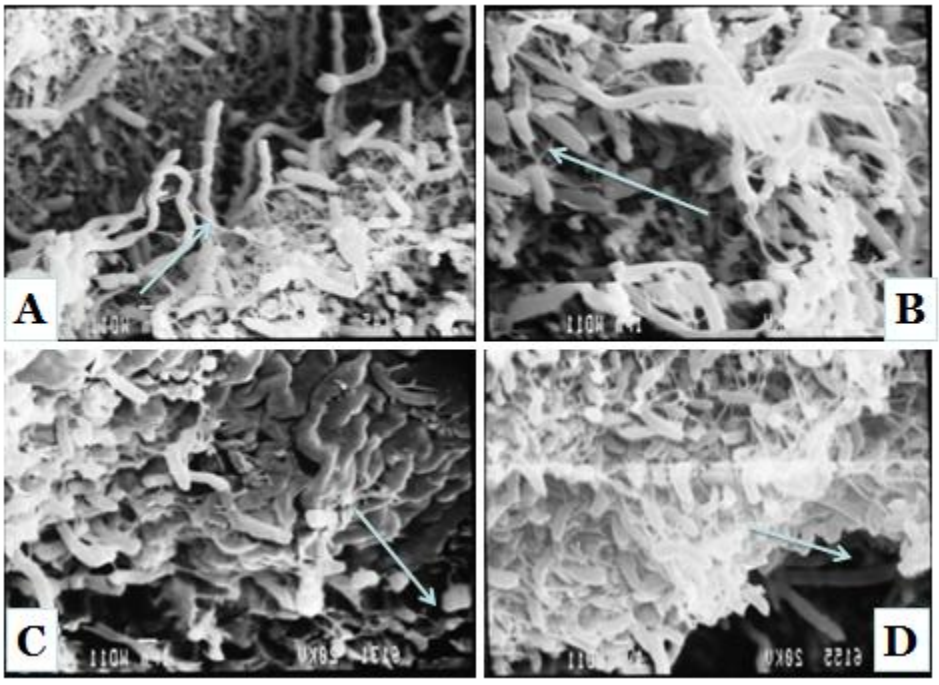


Figure 3.5: Scanning electron microscope (SEM) images; **(**A and B) Depict predominant bacterial morphology of the H_2 producing granules, with arrow showing extracellular polymers for bacterial attachment. (C and D) Shows interior porous structure.

The degree of granular bed expansion was strongly influenced by bacterial biomass density and the degassed effluent recycle rate. Percentage bed expansion at a degassed effluent recycle rate of 3.5 L/min declined sharply with increasing total bacterial granular biomass densities (Figure.3.6). The maximum pumping capacity of the Boyser® Bonfiglioli AMP-16 peristaltic pump for effluent recycling was 3.5 L/min and this restricted the height of bed expansion versus biomass densities to the levels shown in Figure 3.6. Thus % bed expansion declined sharply with increasing total bacterial granular biomass densities.

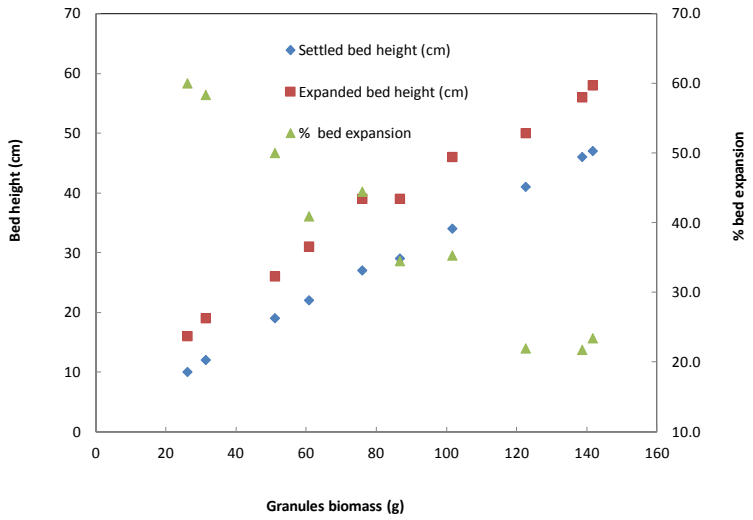


Figure 3.6: Relationship between total bacterial granule biomass per 5 L bioreactor working volume and the following bioreactor granular bed parameters: settled bed height (cm), expanded bed (cm) and % bed expansion.

3.3.2. Total biohydrogen production

The total biohydrogen produced by the bioreactor increased with bacterial granule biomass density (Figure 3.7). Total hydrogen gas flux from the bioreactor rose to 50 L H_2/h after the biomass density had reached of 28.4 g/L. At this biomass density 91% of the sucrose supplied was consumed at a sucrose loading rate of 240.3 g sucrose/h. At this sucrose loading rate the bioreactor generated per g of sucrose consumed was 228.2 ml of H_2 .

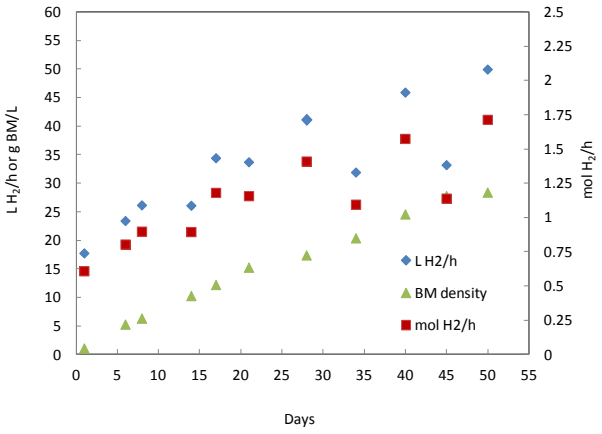


Figure 3.7: Total hydrogen gas flux from the granular bed bioreactor as influenced by biomass density over 50 days. Total hydrogen production expressed in L H_2/L/h and mol H_2/L/h (calculated at ambient pressure of 85 kPa). The degassed effluent recycle rate was 3.5 L/min.

3.3.3. Influence of biomass density on HP

Volumetric hydrogen productivity increased to a maximum level of 9.98 L H_2/L/h or 342.4 mmol H_2/L/h after the biomass density reached 28.4 g/L. Over the biomass density interval between 1.05 g/Land 28.4 g/L *HPs* increased linearly as biomass densities rose (Figure. 3.8).

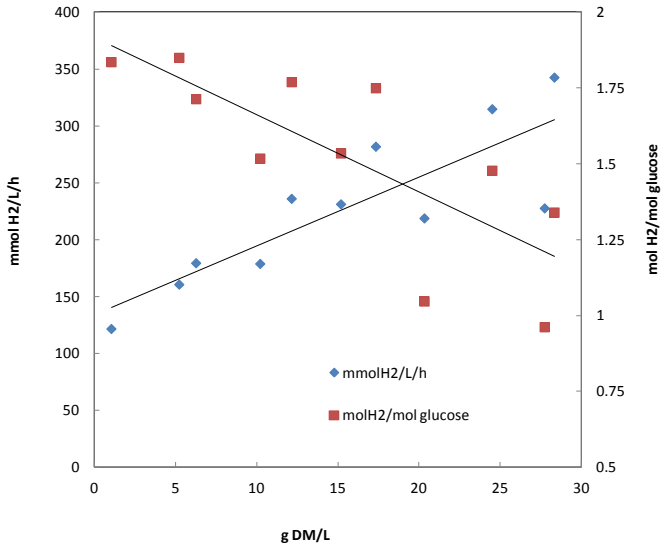


Figure 3.8: The influence of bacterial granule biomass density (g/L) on volumetric hydrogen productivity (*HP*, mmol H_2/L/h) and hydrogen yield (*HY*, mol H_2/mol glucose). The degassed effluent recycle rate was 3.5 L/min

3.3.4. Influence of degassed effluent recycle rate on HP

At a biomass density of 28.4 g/L the *HP* decreased from 342.4 mmol H_2 /L/h to 69.6 mmol H_2 /L/h when the degassed effluent recycle rate was reduced from its maximum of 3.5 L/min to 1.3 L/min (Figure 3.9). Similarly, at a biomass density of 5.2 g/L the HP decreased from 160.4 mmol H_2 /L/h to 31.2 mmol H_2 /L/h as the degassed effluent recycle rate was reduced from its maximum of 3.5 L/min to 1.3 L/min. The linear response trend of *HP* to increasing rates of effluent recycling in Figure 3.9 indicates that *HPs* higher than 342.4 mmol H_2 /L/h could have been achieved had it been possible to increase the effluent recycle rate to values greater than 3.5 L/min.

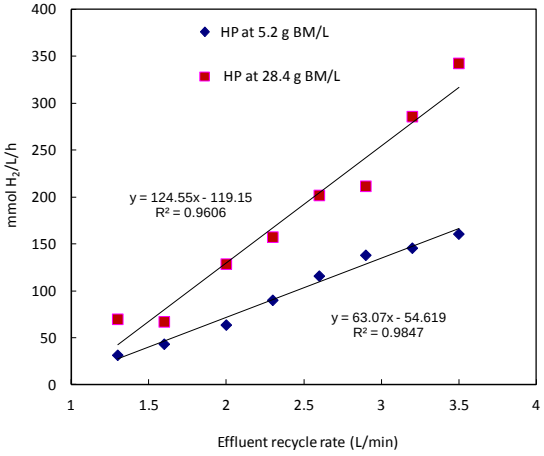


Figure 3.9: Influence of degassed effluent recycle rates on the volumetric hydrogen productivities at two different bacterial granule biomass densities, 5.2 g/L and 28.4 g/L. At a biomass density of 5.2 g/L the nutrient influent rate was 5.4 L/h which corresponded to a sucrose loading rate of 96.1 g/h. At a biomass density of 28.4 the nutrient influent rate was 13.5 L/h which corresponded to a sucrose loading rate of 240.3 g/h.

3.3.5. Influence of biomass density of HY

In contrast to *HP*, *HY* decreased with increasing biomass density (Figure.3.8). At a bacterial granular biomass density of 5.2 g/L, with sucrose loading rate of 96.1 g/h, and a degassed effluent recycle rate of 3.5 L/min, the *HY* reached 1.85 mol H_2/mol glucose. However, at a higher bacterial granular biomass density of 28.4 g/L, with an increased sucrose loading rate of 240.3 g/h, and a degassed effluent recycle rate of 3.5 L/min the *HY* fell to 1.34 mol H_2/mol glucose, representing a 22 % fall in the yield. A negative

correlation coefficient of -0.9612 exists between *HY* and *HP* in Fig. 3.8. In addition a negative correlation coefficient of -0.9664 exists between *HY* and biomass density. This result indicates that the bioreactor's H_2 generating efficiency fell as biomass density increased.

3.3.6. Influence of effluent recycle rate of HY

At a constant biomass density of 5.2 g/L and a constant sucrose loading rate of 96.1 g/h, the HY declined from 1.85 mol H_2/mol glucose to 0.35 mol H_2/mol glucose as the degassed influent recycle rates were reduced from 3.5 L/min to 1.3 L/min (Figure 3.10). Also at a constant biomass density of 28.4 g/L and a sucrose loading rate of 240.3 L/h , the *HY* declined from 1.34 mol H_2/mol glucose to 0.28 mol H_2/mol glucose as the degassed influent recycle rates were reduced from 3.5 L/min to 1.3 L/min (Figure.3.10). As was the case for *HP*, *HY* also increased in linear fashion with increasing degassed effluent recycle rate. This trend also indicates that *HYs* higher than 2.0 mol H_2 /mol glucose for a biomass density of 28.4 g/L could have been achieved had it been possible to increase the effluent recycle rate to values greater than 3.5 L/min. Again, these results confirm the possibility that the bioreactor system was under-performing with respect to the H_2 efficiencies that could otherwise be attained with higher degassed effluent recycle rates.

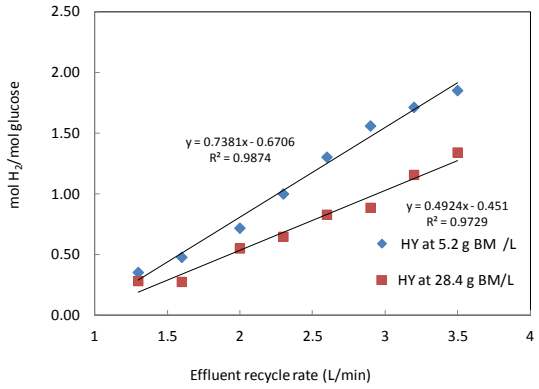


Figure 3.10: Influence of degassed effluent recycle rates on the hydrogen yield (*HY*) measured in mol H_2/mol glucose at two different bacterial granule biomass densities, 5.2 g/L and 28.4 g/L. At a biomass density of 5.2 g/L the nutrient influent rate was 5.4 L/h which corresponded to a sucrose loading rate of 96.1 g/h. At a biomass density of 28.4 the nutrient influent rate was 13.5 L/h which corresponded to a sucrose loading rate of 240.3 g/h.

3.3.7. Specific hydrogen productivity

Also contrary to the trends observed for *HP*, the specific hydrogen productivity had an negative relationship to bacterial biomass density (Figure 3.11). Specific hydrogen productivities fell from 30.68 mmol H_2/g/h (0.89 L H_2/g/h) to 12.08 mmol H_2/g/h (0.35 L H_2/g/h) as biomass density increased from 5.2 g/L to 28.4 g/L. Thus a 5 fold increase in biomass density resulted in a 60% decline in the *SHP* even though the sucrose loading

rate increased from 96.1 g/h to 240.3 g/h. Specific hydrogen productivity was also strongly dependent of the degassed effluent recycle rate (Figure 3.12). At a constant sucrose loading rate of 96.1 g/h and a constant biomass density of 5.2 g/L, *SHP* underwent a 77 % decline from 28.4 to 10.1 mmol H_2/g/h when the degassed effluent recycle rate was reduced to from 3.5 L/min to 1.3 L/min. (Figure 3.12). At a constant sucrose loading rate of 240.3 g/h and a constant biomass density of 28.4 g/L, *SHP* underwent a 77 % decline from 12.08 to 2.81 mmol H_2/g/h when the degassed effluent recycle rate was reduced to from 3.5 L/min to 1.3 L/min. (Figure 3.12). Thus for a given nutrient influent rate and microbial biomass density, the hydrogen production efficiency measured in terms of *SHP* was strongly dependent on the rate of H_2 removal through degassed-effluent-recycling

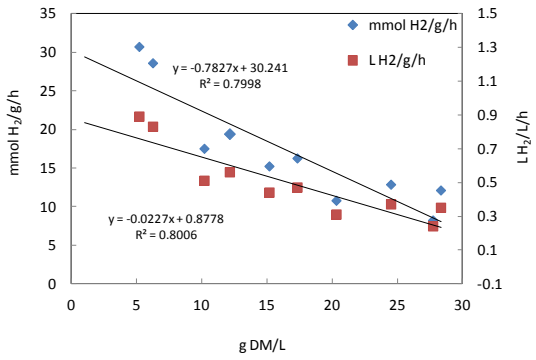


Figure 3.11: Influence of bacterial granular biomass density on the specific hydrogen productivity (*SHP*). The degassed effluent recycle rate was 3.5 L/min.

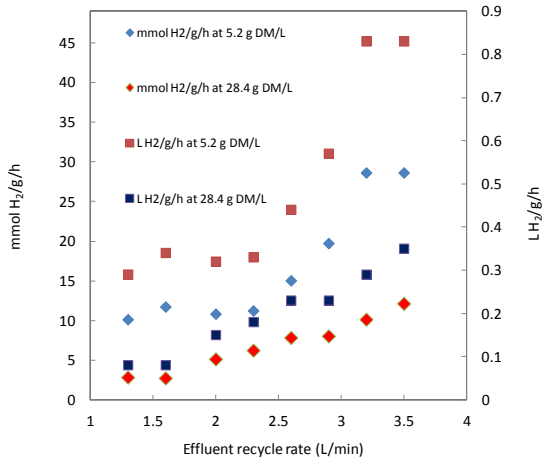


Figure 3.12: Influence of degassed effluent recycle rates on the specific hydrogen productivities in L H_2/g/h and mmol H_2/g/h at two different bacterial granule biomass densities, 5.2 g/L and 28.4 g/L. At a biomass density of 5.2 g/L the nutrient influent rate was 5.4 L/h which corresponded to a sucrose loading rate of 96.1 g/h. At a biomass density of 28.4 g/L the nutrient influent rate was 13.5 L/h which corresponded to a sucrose loading rate of 240.3 g/h.

3.3.8. % H_2 and pH

Both % H_2 content and effluent pH increased in response to increasing degassed effluent recycle rate (Figure 3.13). As degassed effluent recycle rates were increased from 1.3 L/min to 3.5 L/min % H_2 content increased from 49% to 61 %. Similarly, as influent rates were increased from 1.3 L/min to 3.5 L/min the pH increased from 5.1 to 7.2. There

appears to be a correlation or even a biochemical link between the rise in the % H_2 content and an increase in effluent pH.

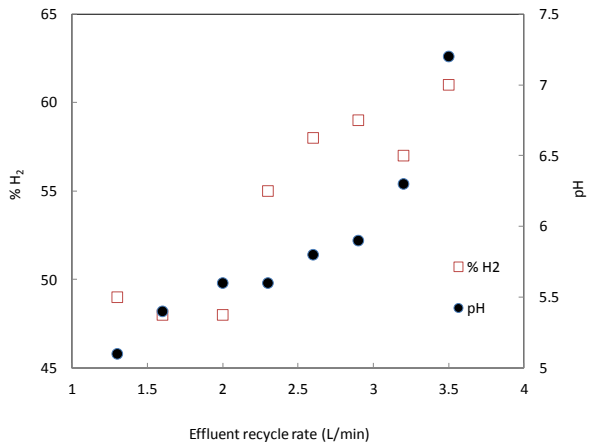


Figure 3.13: Influence of degassed effluent recycle rates on the % H_2 content and effluent pH at a bacterial granule biomass density of 28.4 g/L. The nutrient influent rate was 13.5 L/h which corresponded to a sucrose loading rate of 240.3 g/h.

3.4. Discussion

Maintenance of the highest possible bacterial biomass densities within the *AFGB* remains a fundamental operational goal with respect to maximizing *HPs*. However, increasing bacterial biomass density resulted in a decline in both *HY* and *SHP*. One way to remedy this loss in H_2 production efficiency would be to increase the degassed effluent recycling rate above the maximum 3.5 L/min level used in this study. A volumetric flow of 3.5 L/min through the bioreactor corresponds to a linear upward fluid velocity of 1.16 cm/s.

The average granule settling velocity was 3.5 cm/s or 3 times greater than the recycled effluent up flow linear velocity. Thus a 3 fold increase (10.5 L/min) in degassed effluent recycle rate could have been applied to the current bioreactor design without any significant washout of granule biomass occurring. Assuming that a constant linear increase in *HY* would occur in response to further incremental increases in the effluent recycle rate above the 3.5 L/min rate, then in theory an *HY* of 4.72 mol H_2/mol glucose could be attained with an increase in the effluent recycle rate to say 10.5 L/min. This claim is justified in the conclusion below and also in Chapter 6. Again assuming that a constant linear increase in *HP* would occur following the incremental increase of the effluent recycle rates above 3.5 L/min, then by increasing the degassed effluent recycle rate above the 3.5 L/min threshold a simultaneous increase in both *HP* and *HY* with increasing bacterial biomass densities could be expected.

3.5. Conclusion: 'Can the Thauer Limit' be exceeded ?

Strictly speaking the concept of the Thauer Limit of a maximum of 4 mol H_2/ mol glucose only applies to dark anaerobic fermentation based on a bacterial monocultures. For a multispecies bacterial consortium it possible for the so-called Thauer Limit to be exceeded because of the action of secondary proton reducing syntrophic bacteria. So in principle the concept of the Thauer Limit has only limited and narrow applicability.

Hydrogen is an important intermediate in the dark anaerobic oxidation of organic compounds to CO_2 and CH_4. Anaerobic oxidation of organic compounds depends on the cooperative interactions between the following function groups of bacteria:

1) the primary fermentation bacteria;
2) the secondary proton reducing syntrophic bacteria;
3) acetogenic bacteria;
4) two types of methanogenic archaea bacteria.

The primary fermentation bacteria include hydrolytic bacteria that degrade the various biopolymers such as proteins, polysaccharides, nucleic acids and lipids to monomers such as amino acids, sugars, purines, pyrimidines, long chain fatty acids. The group of primary fermentation bacteria also include acidogenic bacteria that ferment the monomers to alcohols, short chain fatty acids, organic acids, CO_2 and H_2. The secondary proton reducing syntrophic bacteria oxidize alcohols and short chain fatty acids to acetate, CO_2 and H_2. Syntrophic bacteria also exist which oxidize acetate to CO_2 and H_2. Acetogenic bacteria synthesize acetate from CO_2 and H_2.

In nature the oxidation of ethanol, acetate, butyrate, and propionate into CO2 and H_2 is facilitated by interspecies hydrogen transfer between the secondary proton reducing syntrophic bacteria and the H_2 consuming hydrogenotrophic methanogens. In the absence of methanogenesis the oxidation of alcohols such ethanol, and short chain fatty acids such as, acetate, butyrate and propionate into CO_2 and H_2 by the secondary syntrophic proton reducing bacteria becomes thermodynamically unfavourable.

High rates of H_2 stripping has been shown to promote the oxidation of ethanol and short chain fatty acids. For example, sparging with N_2 promoted the anaerobic oxidation of alcohols and short chain fatty by proton reducing syntrophic bacteria in the absence of H_2 consuming hydrogenotrophic methanogens (Valentine *et al*., 2000;

Adams *et al*., 2006). Phase partitioning of H_2 has been shown to decrease the propionate concentration in a upflow anaerobic sludge bed (UASB) bioreactor (Frigon and Guiot, 1995). Normally in UASB bioreactors propionate concentration increases with increasing dissolved H_2 concentrations. Recycling of biogas through the sludge bed decreased both the concentration of propionate and dissolved H_2 in the sludge bed (Frigon and Guiot, 1995). The recycle biogas also contained H_2. Biogas recycling directly into the bulk liquid phase of UASB increased the transfer of H_2 from the liquid to gas phase by increasing the interfacial specific area $k_L a$ between the two phases. This process facilitated propionate degradation even though the lowest dissolved H_2 value recorded (0.44 µm) was still 6.0 fold higher than the thermodynamic threshold concentration for propionate oxidation (0.074 µm) (Frigon and Guiot, 1995). In the above UASB H_2 phase partitioning experiment it was proposed that propionate had been oxidized at a faster rate than its production as the biogas recycling rate was increased. The interfacial specific area for dissolved gas partitioning was defined as follows:

$$k_L a = \frac{Q_v}{K_H RT \left(\frac{[H_2]_L}{[H_2]_L^*} - 1\right)} \qquad (3.4)$$

Where, $[H_2]_L$ is the super saturated concentration (mol/L) of dissolved H_2 in the bioreactor bulk liquid phase, $[H_2]_L^*$ is the concentration (mol/L) of dissolved H_2 in the bioreactor bulk liquid phase at thermodynamic equilibrium according to Henry's law, Q_v

is the volumetric gas production rate (L/L/d) , K_H is Henry's law constant for H_2 , R is the ideal gas constant, T is temperature (K).

Liquid-to-gas phase mass balance dynamics for H_2 assumes an equilibrium distribution of H_2 based on Henry's law. However, the low solubility and low mass transfer coefficients of gases like H_2 can delay the attainment of thermodynamic equilibrium between the different phases of the anaerobic fluidized granular bed bioreactor (*AFGB*) system (Obazu *et al*., 2012). For example, gaseous H_2 fluxes from the *AFGB* system involves H_2 mass transfers between three different material phases which also happen to be in different states of motion. In the *AFGB* system we have the co-existence of a quasi-static solid phase, a mobile bulk fluid or liquid phase and a mobile gaseous phase. The quasi-static solid phase consists of the fluidized bacterial granular bed which functions as the H_2 generating biocatalyst. The mobile liquid phase consists of the nutrient influent and recycled degassed effluent. The mobile gaseous phase consists of gas bubbles generated through the process of cavitation or bubble nucleation within the bioreactor. Increasing the rate of degassed effluent recycling through the fluidized granular bed will increase the rate of bubble production, thereby also increasing the interfacial specific area $k_L a$ for H_2 gas exchange between the liquid phase and gaseous phase within the fluidized bed.

Rapid partitioning of H_2 from the liquid phase into an expanding gaseous phase will shift all anaerobic oxidation processes within the bacterial granules away from the thermodynamic equilibrium. In addition, the high upward linear velocities of the two mobile phases will further contribute to the shifting of the anaerobic oxidation processes within the granules away from the thermodynamic equilibrium state through the physical

removal of H_2 from the quasi-static solid phase. We propose that the combined effects of:

1) rapid partitioning of H_2 into the gaseous phase by increasing $k_L a$;
2) rapid physical removal of H_2 through the high upward flow velocity of the liquid phase;
3) rapid physical removal of H_2 through the high upward flow velocity of the gaseous phase;

create physical conditions within fluidized bed which are similar to the conditions that promote anaerobic VFA oxidation in the absence of methanogenesis (Valentine *et al*., 2000; Adams *et al*., 2006). Thus if anaerobic VFA oxidation were also favoured by the combined effects of the above processes 1), 2), and 3) , then the Thauer Limit of 4 mol H2/mol glucose which is normally applied for the reaction involving the anaerobic oxidation of glucose to hydrogen acetate would be exceeded and would not be application for a mixed bacterial culture in an *AFGB* system. This support the prediction that by extrapolation of the linear relationship in Figure 6.2 in chapter 6 **a** *HY* exceeding 4.0 mol H_2/mol glucose would be attainable under thermophilic temperatures.

CHAPTER FOUR

STABILITY OF BIOHYDROGEN PRODUCTION AT EXTREME THERMOPHILIC (70^{O}C) TEMPERATURES BY AN UNDEFINED BACTERIAL CULTURE

4.1 Introduction

Commercial exploitation of biohydrogen depends on increasing the space/time yields (*STYs*) per unit volume for biohydrogen production above some objective *STY* threshold. One example of an *STY* threshold would be the minimum acceptable volumetric supply rate of H_2 necessary for driving electricity generation from a 5 kW fuel cell. It has been estimated that a 5 kW fuel cell would require an H_2 supply rate of 2900 L H_2 /h (Levin *et al*., 2004). From an *STY* perspective this would be equivalent to a volumetric hydrogen production rate of 2.9 L H_2/(L.h) or 120 mmol H_2 /(L.h), irrespective of hydrogen yield (mol H_2/mol glucose). Volumetric hydrogen productivities (*HPs*) ranging from 7.3 L H_2/(L.h) to 14.8 L H_2/(L.h) with hydrogen yields (*HYs*) not exceeding 2.2 mol H_2/mol glucose have been achieved for mesophilic and thermophilic anaerobic fluidized bacterial granular bed bioreactors (Lee, *et al*., 2004; Lee, *et al*., 2006; Ngoma, *et al*., 2011). Thus while bioreactor *HP* capacities have been shown to exceed the above *STY* threshold by 2.5 to 5 fold, *HYs* remain below 3.0 mol H_2/ mol glucose, and maintenance of volumetric bacterial biomass densities often result bioprocess stability problems.

High volumetric bacterial biomass densities have been produced through the formation of bacterial biofilm on a suitable carrier surface or through bacterial granulation (Zhang *et al*., 2008b). Either way, high volumetric bacterial biomass densities

(26 to 40 g/L) have facilitated the achievement of volumetric biohydrogen productivities greater than 2.9 L H_2/(L.h) (Lee *et al*., 2006; Thompson *et al*., 2008; O Thong *et al*., 2008; Zhang *et al*., 2008b; Shida *et al*., 2009; Barros *et al*., 2010; Ngoma *et al*., 2011). However, while biofilm coated particles or bacterial granules have good settling properties which ensure bacterial biomass retention within the bioreactor at high dilution rates, high rates of biomass washout in bench scale bioreactors still occur when biogas becomes trapped in gas filled cavities within the biofilm or granular bed. At low hydraulic retention times, for example, 0.5 h for a bioreactor volume less than 1.0 L (see Table 4.1), the accumulation of large gas bubbles or gas slugs within the expanded or fluidized bed causes a *sludge piston floatation* process (Lee *et al*., 2006) which can result in the complete washout of the entire granular bed from the bioreactor. This major instability problem associated with high *HP* bacterial granular bed bioreactors can be prevented by either mechanical agitation via an impellor inserted into the fluidized granular bed (Lee *et al*., 2006) or by fitting a sedimentation column above the expanded or fluidized granular bed (Zhang *et al*., 2008a,b). Either option increases the long term stability of a bench scale bioreactor operation as bacterial biomass densities increase above 20 g/L.

In this study the influence of a sedimentation column on the operational stability of a high rate extreme thermophilic (70 ^{o}C) fluidized granular bed bioreactor system was investigated.

4.2 Materials and Methods

4.2.1 Medium

An Endo formulation (Endo *et al*., 1982; Thompson *et al*., 2006) was used as the nutrient medium for inoculum preparation and for the bioreactor experiments. The medium contained 17.8 g sucrose/L and the following mineral salts (g. L^{-1}): NH_4HCO_3 6.72, $CaCl_2$ 0.2, K_2HPO_4 0.699, $NaHCO_3$ 3.36, $MgCl_2.6H_2O$ 0.015, $FeSO_4.7H_2O$ 0.0225, $CuSO_4.5H_2O$ 0.005, and $CoCl_2.H_2O$ 1.24 x 10^{-4}g.

4.2.2. Inoculum preparation

An undefined extreme thermophilic anaerobic bacterial consortium was derived from a mixture of sewage sludge and fresh cow dung. Sewage sludge was obtained from the overflow outlet of a mesophilic anaerobic digester at the Olifantsvlei wastewater treatment works (Johannesburg). Fresh cow dung was obtained from grass fed dairy cows at the Animal and Dairy Research Institute (Irene), Gauteng. Sewage and dung samples were incubated in Endo medium (50% v/v) at 90 ºC for 2 hours. After the heat treatment the pH of the samples were reduced to pH 2.0 with 0.1 N HCl. Inoculum samples were kept at this pH in sealed airtight Schott bottles for 12 h at room temperature and then readjusted to pH 7.0 by mixing with Endo medium (50% v/v). The two inoculum preparations, sewage (1 L) and dung (1 L) were then applied to the bioreactor.

4.2.3. Bioreactor design and set-up

The bioreactor system consisted of the following 4 components: an influent and recycled effluent inlet manifold or diffuser, tubular bioreactor, a liquid-solid separator or

sedimentation column connected to the top end of the tubular bioreactor and a tubular gas-disengager (Figure 3.1). Clear Perspex hollow tube was used for the construction of the tubular bioreactor (internal diameter (ID): 80 mm; height (H): 1000). The working volume for the tubular bioreactor's fluidized bacterial granular bed was 5 L. Volumetric hydrogen productivity was expressed in term of this volume rather than the total working volume of the bioreactor system. An 11.6 L liquid-solid separator was connected to the top end of the tubular bioreactor for solid-liquid separation to prevent the washout of the granules from bioreactor, especially at high effluent recycle rates. The solid - liquid separator consisted of two parts a 5.3 L component (ID: 150 mm and H: 300 mm) and a 6.3 L component (ID: 200 mm and H: 200 mm). At the base of the bioreactor the clear Perspex cylinder was connected to a conical shaped diffuser (ID: 80 mm and H: 150 mm) made from PVC which functioned as the primary inlet for the effluent recycle stream. A stainless steel sieve (32 mesh) was fixed over the inlet of the diffuser. Above the stainless steel sieve the conical diffuse was filled with a 100 mm layer of 5 mm glass beads. Positioned at the upper end of the diffuser were 4 inlet ports (ID 5 mm) with each inlet arranged at 90^{o} with respect to the two other inlets on each side. Nutrient medium (influent stream) was supplied directly into the upper glass bead layer via the 4 inlet ports. The effluent overflow from solid-liquid separator was decanted into a gas-disengager which consisted of a gas collection cylinder (H: 200 mm and ID: 150 mm) connected to a gas-disengager cylinder (H: 600 mm and ID: 60 mm). The gas-disengager had two effluent outlets, one at the bottom that was connected to a variable Boyser® Bonfiglioli AMP-16 peristaltic pump (0.37 kW) which was used to recycle de-gassed effluent into the bioreactor via the diffuser. For effluent recycling the pump was set

between 15 rpm and 50 rpm which gave a volumetric pumping rate ranging from 1.3 L/min to 3.5 L/min. The second effluent outlet drained the excess effluent overflow from the gas-disengager. The gas-disengager gas-outlet port was connected to a gas meter (Ritter drum-type gas meter TG 05/3). All Ritter drum gas meter measurements were carried out 25^{o}C. The liquid-gas separator or gas-disengager had a working volume of 1.54 L and the total fluid occupied volume of the interconnecting piping was 1.9 L. Total fluid containing volume of the bioreactor system (bioreactor bed, solid-liquid separator, gas-disengager, diffuser, and piping) was 20.0 L. Bioreactor and gas-disengager temperatures were maintained at the two operational temperatures, 60 ºC and 70 ºC, by circulating heated water from a heated water bath through the bioreactor and gas-disengager water jackets. A Watson-Mallow (model 520U) peristaltic pump (Falmouth, UK) was used to pump the Endo nutrient into the bioreactor.

4.2.4. Bacterial granule induction

On top of the glass bead bed a 100 mm bed of cylindrical activated carbon (*CAC*) particles (diameter = 2.5 mm and length = 5.0 mm) was used to facilitate the induction of bacterial granulation in the bioreactor (Lee *et al*., 2004). Prior to its use, the *CAC* was first washed with distilled water to remove all the suspended fine particles and then sterilized by autoclaving for 20 minutes. Concentrated (3x) Endo medium (18.0 L) and seed inoculum (2.0 L) was added to the bioreactor system. Following inoculation the bioreactor was operated on a batch effluent-recycle mode for 48 h at 70^{o}C to acclimatize the bacteria and allow for their attachment to the *CAC*. After this acclimatization period the bioreactor operation was switched to continuous – effluent recycle mode with an

initial hydraulic retention time (*HRT*) of 8 h, supplying Endo medium at its normal concentration. The *HRT* was then gradually decreased over 2 day intervals by increasing the nutrient medium supply rate. As the *HRT* was decreased from 8 to 4 h the growth and development of bacterial biofilm on the *CAC* particles became visible. With further decreases in the *HRT* below 4 h the biofilm growth increased and bacterial granules began to form and accumulate at the surface of the expanded *CAC* bed. Once granule formation had been initiated, further reductions in the *HRT* to between 2 and 1.6 h resulted in the rapid growth and expansion of the granular bed. Granule induction, initial growth and initial development were carried out at 70^{o}C.

4.2.5. Effluent recycle rate and effluent gas disengagement

The effluent discharged from the bioreactor was passed through a gas-disengager before being recycled back into the bioreactor (Ngoma *et al*., 2011). Effluent discharge force into the gas-disengager was dependent on the effluent recycle rate. High rates of effluent recycling between the bioreactor and the gas- disengage generated a high degree of fluid turbulence and cavitation within the gas disengager tube. This vigorous mixing process within the gas-disengager facilitated the release of undissolved H_2 from the effluent through bubble production. Efficient removal of undissolved or non-solubilized H_2 trapped in the effluent phase by gas disengagement was expected to increase the overall biohydrogen production efficiency of the bioreactor system (Ngoma *et al*., 2011).

4.2.6. Analytical techniques

Gas chromatography was used to analyze % gas composition (H_2, CO_2 and CH_4). A Clarus 500 GC PerkinElmer equipped with a thermal conductivity detector was used. The temperatures of injector, detector and column (PerkinElmer Elite Q Plot capillary column 30 m x 32 mm) were kept at 250 °C, 200 °C and 45°C, respectively. Argon was used as the carrier gas at a flow rate of 2.0 ml min^{-1}. Sample gas injection volume was 40µl. The following formula (equation 4.1) was used for converting total bioreactor gas flux (L/h) to mmol H_2/h ,

$$\frac{\Delta H_2}{\Delta t} = \frac{P\left[\left(\%H^{GC}\right)\frac{\Delta V}{\Delta t}\right]}{RT} \qquad (4.1).$$

Where, $\Delta H_2/\Delta t$ = mmol H_2 /h; P = atmospheric pressure (85 kPa); ($\%H_2^{GC}$) = percentage hydrogen content from GC measurements; $\Delta V/\Delta t$ = L/h of total gas production from the gas meter measurements; R is the gas constant (8.314 J/(K.mol)); T = 298.15 K (the temperature at which the gas flow from the bioreactors were monitored).

The concentration of sucrose in the bioreactor influent and effluent streams was determined using the sucrose-resorcinol method (Kerr *et al*., 1984).

4.2.7. Experimental design

After granule formation was initiated the influent rate for the duration of the experiment was maintained at 5.4 L/h at night (14 h). During the day (10 h) the influent rates were maintained at the following rates for between 3 and 7 days: 5.4 L/h, 6.3 L/h, 7.2 L/h, 8.1 L/h, 9.0 L/h, 9.9 L/h, 10.8 L/h, 11.7 L/h, 12.6 L/h and 13.5 L/h. The bioreactor was

operated in such a fashion for 50 days. While the substrate used was sucrose, hydrogen yield (*HY*) has been expressed in terms of glucose in the following units mol H_2/mol glucose.

Hydrogen gas production and sucrose consumption measurements were determined for each of the above day time influent rates. Measurements were first carried out when the bioreactor was operated at 70 ^{o}C. The temperature of the bioreactor was then dropped to 60 ^{o}C and allowed to acclimatize at this temperature for 5 h before hydrogen and sucrose consumption measurements were undertaken. All gas and sucrose measurements were replicated three times.

4.3. Results and discussion

4.3.1. Granule growth and bioreactor operation stability

Following the inoculation of the bioreactors granule formation took place within 5 days after the Endo supply rate or influent rate had reached 5.4 L/h. To grow the granular bed the influent rate was then increased every 3 to 7 days. At the end of 50 days the settled bed height of the granule bed had grown to 45 cm corresponding to a total bacterial dry mass of 135 g (Figure 4.1). After 50 days the biomass density reached 27 g/L for the 5 L bioreactor. The 11.6 L liquid-solid separator prevented gas sludge piston induced granular bed washout during the 14 h night period when the bioreactor was operated with an influent rate of 5.4 L/h and a degassed effluent recycle rate of 3.5 L/h. Also, during the day period the granule bed remained stable within the bioreactor at all influent rates and also at all degassed effluent recycle rates for the full 10 h diurnal operation time.

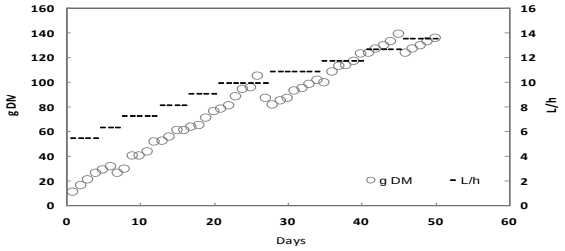


Figure 4.1: Bacterial granular growth in response to influent supply rate.

4.3.2. Influence of temperature and biomass density on biohydrogen productivity

At 70 ^{o}C total biohydrogen production increased with granule biomass, producing up to 49.9 L H_2/h (Figure 4.2A), which corresponded to a volumetric hydrogen productivity of 9.98 L H_2/L/h or 342.4 mmol H_2/L/h, with a sucrose consumption of 91 %. These values are similar to the ones (Table 4.1) achieved for a fluidized granular bed system that was agitated with an impeller (Lee *et al*., 2006). Specific hydrogen productivity (*SHP*) fell from 30.68 mmol H_2/g/h (0.89 L H_2/g/h) to 12.08 mmol H_2/g/h (0.35 L H_2/g/h) as biomass density increased (Figure 4.3A). Reported *SHPs* also tend to be variable (Table 4.1) , ranging from 0.113 to 0.283 L H_2/g/h for the CIGSB systems (Lee *et al*., 2006) or from 4.18 to 9.53 mmol H_2/g/h for biofilm and granular sludge AFBR systems (Zhang *et al*., 2007, 2008)

Table 4.1: Summary of bioreactor parameters and hydrogen productivity variables for different high performance bioreactor systems.

Bioreactor system	Substrate	Concent-ration	T	HRT	pH	%H2	HP	HP	HY	SHP	SHP	B V	B D	References
		g/L	^{o}C	h			L H_2/L/h	mmol H_2/L/h	mol H_2/ mol glucose	L H_2/g/h	mmol H_2/g/h	L	g/L	
CSTR FLOC	glucose	10 2.5 2.5	30 30 30	1 10 2.5	5.5 5.5 5.5	61 67 72	2.17 0.081 0.27		1.7 2.8 2.4			2.0 2.0 2.0		Van Ginkel and Logan, 2005
AFBR GAC		10 10 20 30	37 37 37 37	0.5 1.0 1.0 1.0	4.0	57.2 59.2 59.2 59.2	2.22 2.36		1.16 1.19 1.10		4.18 4.34	0.6	21.5 18.7 21.6	Zhang *et al.*, 2007
BF AFBR	glucose	10	37	0.25	5.5		7.6		1.71		8.96	0.6[a] 1.4[b]	61 – 65[c] 34 – 37[d]	Zhang *et al.*, 2008a; Zhang *et al.*, 2008b
GS AFBR	glucose	10	37	0.25	5.5		6.6		1.66		8.77	0.6[a] 1.4[b]	61 – 65[c] 34 – 37[d]	Zhang *et al.*, 2008a; Zhang *et al.*, 2008b
UASB	sucrose	20	60	0.75	5.0	42	3.72	152.5	1.3		9.53	0.22	16	O Thong *et al.*, 2008
AFBR	glucose	4	30	1.0	5.5	51	1.21		2.52			4.19		Barros *et al.*, 2010
AFBR	glucose	2	30	2.0	3.8	37	1.28		2.29			4.19		Shida *et al.*, 2009
CIGSB SAC	sucrose	17.8	35	0.5	6.7	38.1	7.33		1.52	0.280		1.0	26.1	Lee *et al.*, 2004
CIGSB CAC	sucrose	17.8	35	0.4	6.7	35.6	7.06		1.19	0.283		1.0	26.1	
CIGSB	sucrose	17.8	30	0.5	6.7	34.9	3.93		1.19	0.113		0.88	30 - 40	Lee *et al.*, 2005
CIGSB	sucrose	17.8	35	0.5	6.7	40.5	6.87		1.56	0.189		0.88	30 - 40	
CIGSB	sucrose	17.8	40	0.5	6.7	40.1	7.66		1.58	0.223		0.88	30 - 40	
CIGSB	sucrose	17.8	45	0.5	6.7	32.9	5.28		1.33	0.174		0.88	30 - 40	
CIGSB	sucrose	17.8	35	0.5	6.7	40.5	6.87		1.56	0.187				Lee *et al.*, 2006
CIGSB[e]	sucrose	17.8	35	0.5	6.7	41.7	9.31	380 (at 101.3 kPa)	1.96	0.234	9.5		40	Lee *et al.*, 2006
AFGB	sucrose	17.65	37	4.5	6.2	42.3		180 (at 85 kPa)	1.17			2.89		Thompson *et al.*, 2008
AFGB HER	sucrose	17.8	45	0.37	5.4	45	8.71	296 (at 85 kPa)	1.24	0.491		5.0[b] 10.5	19.5	Ngoma *et al.*, 2011
AFGB HER	sucrose	17.8	70	0.37	5.5	67	14.8	506 (at 85 kPa)	2.2	0.724		5.0[b] 10.5[f]	22.7	Ngoma *et al.*, 2011
AFGB HER	sucrose	17.8	60	0.37	7.2	60	7.86	270 (at 85 kPa)	0.92 to 1.66	0.29	9.95	5.0[b] 19.1[f]	28.35	Current study
AFGB HER	sucrose	17.8	70	0.37	7.2	68	9.98	342.4 (at 85 kPa)	1.34 to 1.85	0.35	12.08	5.0[b] 19.1[f]	28.35	Current study

AFBR: anaerobic fluidized bed; AFBR GAC: AFBR with bacteria biofilm attached to granulated activated carbon; *AFGB* HER: anaerobic fluidized granular bed reactor with high rate effluent recycling; AFBR : biofilm anaerobic fluidized bed reactor; CIGSB *CAC*: carrier induced granular sludge bed with cylindrical activated carbon; CIGSB SAC: with spherical activated carbon; AFBR granular sludge anaerobic fluidized bed; UASB: upflow anaerobic sludge bed; CSTR FLOC: continuous stirred tank reactor with bacterial flocs. a: bioreactor working volume corresponding to bed biofilm or granular height; b: total bioreactor working volume; c: biomass in corresponding to bed height; d: biomass in total bioreactor working volume; e: CIGSB with bed agitation; f: total bioreactor system volume.

On day 50, for a settled granular bed height of 45 cm, when the degassed effluent recycle rate was reduced from 3.5 L/min to 1.3 L/min the *HP* also fell from 342.4 mmol H_2 /L/h to 69.6 mmol H_2 /L/h (Figure 4.4A). Similarly, *SHP* dropped from 12.08 to 2.81 mmol

H_2/g/h when the degassed effluent recycle rate was reduce to from 3.5 L/min to 1.3 L/min. (Figure 4.4B). Reported mesophilic *HPs* range from 1.21 to 9.31 L H_2/L/h (Table 4.1). Thermophilic *HPs* range from 3.72 L H_2/L/h, calculated from the 152 mmol H_2/L/h value reported by O-Thong *et al*., 2008, to 14.8 L H_2/L/h (506 mmol H_2/L/h) reported by Ngoma, *et al*., 2011 (Table 4.1).

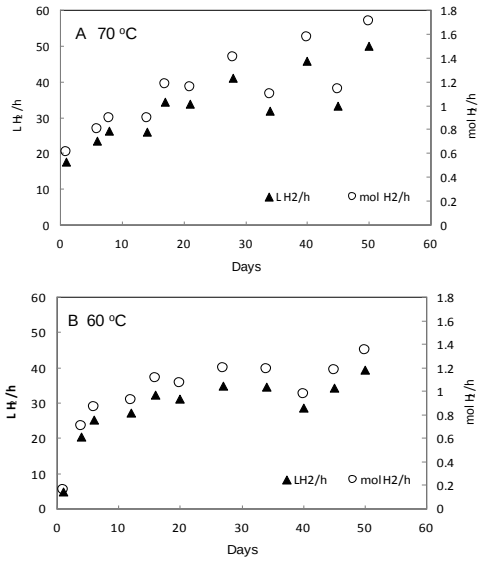


Figure 4.2: Total hydrogen production under extreme thermophilic and thermophilic conditions with respect to granular bed growth over 55 days (see Figure 4.1). **A.** Total hydrogen production in terms of L H_2/L/h and mol H_2/L/h (calculated at ambient pressure of 85 kPa) at 70^{o}C. **B.** Total hydrogen production in terms of L H_2/L/h and mol H_2/L/h (calculated at ambient pressure of 85 kPa) at 60^{o}C .

Usually when the degassed effluent recycle rate is reduced the likelihood for gas sludge piston induced granular bed washout increases. However, due to the solid-liquid separator (Figure 3.1) no granular bed wash occurred at the lower effluent recycle rates.

A 10 ^{o}C drop in the temperature from the extreme thermophilic temperature of 70 ^{o}C to a thermophilic temperature of 60 ^{o}C resulted in a substantial 21.4 % decline in the total biohydrogen production, that is, from approximately 50 L H_2/h to 39.3 L H_2/h (Figure 4.2 B). Similarly, a drop in the temperature to 60 ^{o}C also resulted in substantial declines in the *HP* and *SHP* relative to the 70 ^{o}C treatment (Figures 4.3 and 4.4), also indicating that the physiology and biochemistry of the bacterial consortium in the granules had become preferentially acclimatized to life at a extreme thermophilic temperature.

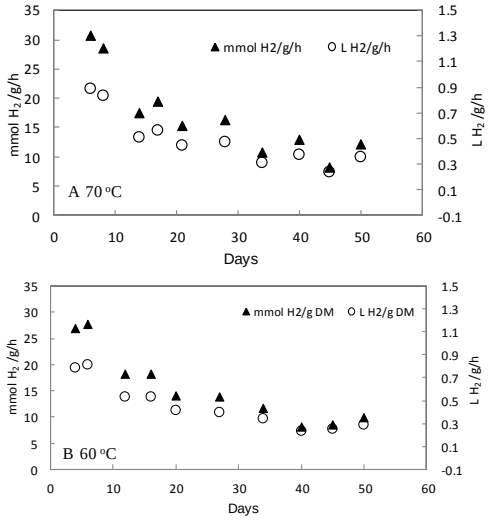


Figure 4.3: Changes in the specific hydrogen productivity with respect to granular bed

growth over time (see Figure 4.1) at extreme thermophilic and thermophilic temperatures .Measurements given in terms of L H_2/g/h and mol H_2/g/h, were g is the bacterial granular biomass. **A.** Specific hydrogen productivity at extreme thermophilic temperatures. **B.** Specific hydrogen productivity at thermophilic temperatures.

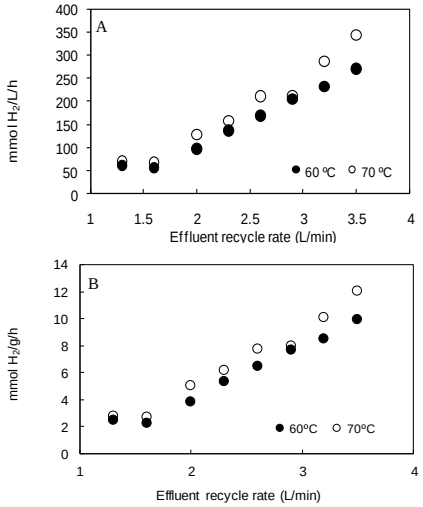


Figure 4.4: Influence of effluent recycle rate on volumetric hydrogen productivity (**A**) and specific hydrogen productivity (**B**) at a granular biomass density of 27 g/L at extreme thermophilic and thermophilic temperatures.

4.3.3. HYs at extreme thermophilic temperatures

In contrast to *HP*, *HY* followed the same trend as *SHP* by also decreasing with increasing biomass density (Figure 4.5). At a low bacterial granular biomass densities of 6.4 g/L, with an influent rate of 6.3 L/h, and a degassed effluent recycle rate of 3.5 L/min, the *HYs*

were 1.66 mol H_2/mol glucose and 1.71 mol H_2/mol glucose, at 60 ^oC and 70 ^oC, respectively (Figure 4.5A). At a higher bacterial granular biomass density of 27 g/L, with an influent rate of 13.2 L/h, and a degassed effluent rate of 3.5 L/min, the *HYs* fell to 1.15 mol H_2/mol glucose and 1.34 mol H_2/mol glucose, 60 ^oC and 70 ^oC, respectively (Figure 4.5B). In addition, irrespective of the granular biomass densities, all *HYs* fell as degassed influent recycle rates were reduced. A reduction in the temperature to 60 ^oC resulted in a slight decrease in *HYs* at all degassed effluent recycle rates (Figure 4.5) . The average *HY* (n =12) at 70 ^oC and 60 ^oC were 1.54 ± 0.29 mol H_2/mol glucose and 1.34 ± 0.24 mol H_2/mol glucose, respectively. For fluidized bed systems, reported mesophilic *HYs* range from 1.10 to 2.52 mol H_2/mol glucose (Table 4.1). For a CSTR with a mixed bacterial floc culture an *HY* of 2.8 mol H_2/mol glucose was achieved at mesophilic temperatures (Van Ginkel and Logan, 2005, see Table 4.1).

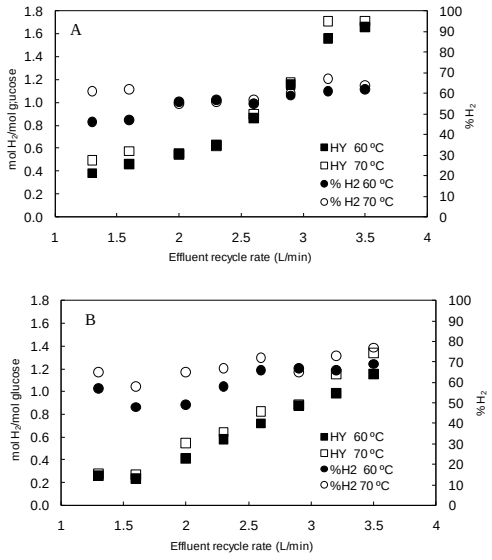


Figure 4.5: Influence of bacterial granular biomass density and effluent recycle rate on hydrogen yield and % H_2 content at extreme thermophilic and thermophilic temperatures. **A**. *HY* and % H_2 at influent rate of 6.3 L/h and bacterial biomass density of 6.3 g/L. **B.** *HY* and % H_2 at influent rate of 13.2 L/h and biomass density of 27 g/L.

4.3.4. % H_2 and pH at hyperthermophilic temperatures

At 70 ^oC the % H_2 content ranged from 60 % to 77%, whereas at 60 ^oC the % H_2 content fell between 60% and 65%, rarely rising above 70% (Figure 4.5). It was also interesting to observe that when the pH of the effluent fell below 6.0 the % H_2 content also always fell below 60% (data not shown). In general the relationship between % H_2 content and pH shows considerable variability (Table 4.1). For example, at mesophilic temperatures (30 to 45 ^oC) the following % H_2 contents and corresponding pHs have been reported (Table 4.1): 72 % H_2 at pH 5.5 (Van Ginkel and Logan 2005); 59.2% H_2 at pH 5.5 (Zhang *et al*., 2007 IJHE); 51% H_2 at pH 5.0 (Barros *et al*., 2010); 37 % H_2 at pH 3.8 (Shida *et al*., 2009); 32.6 to 41.7 % H_2 at pH 6.7 (Lee *et al*., 2004, 2005, 2006); 42.3% H_2 at pH 6.2 (Thompson et al., 2008); 45% H_2 at pH 5.4 (Ngoma *et al*., 2011). At thermophilic temperatures (50 to 60 ^oC) for high *HP* bioreactor processes the following trends have been reported (Table 4.1): 42 % H_2 at pH 5.0 (O-Thong *et al*., 2008); 67 % H_2 at pH 5.5 (Ngoma *et al*., 2011).

4.4 Conclusions

Complete stability of high rates of biohydrogen production was achieved for 50 days continuous operation. Also, stability of pH was maintained without any additional pH

control through acid or base titration. Table 4.1 gives an accurate summary of the current state of the art of high *HP* bioprocesses. What has become increasing clear is that % H_2 is highly variable for mixed bacterial cultures, ranging from 32.7 to 77 %. However, in our studies the % H_2 increased with increasing temperature, and in addition, the highest % H_2 contents were achieved at pHs that did not deviate far from 7.0.

CHAPTER FIVE

BIOHYDROGEN PRODUCTION BY BACTERIAL GRANULES ADAPTED TO GROW AT DIFFERENT THERMOPHILIC TEMPERATURES

5.1Introduction

For thermophilic biohydrogen production to be commercially viable a number of process goals need to be satisfied. Production of net positive work by a thermophilic H_2 generating system would be the most obvious process goal. An energy balance model for computing the net work done under thermophilic temperatures with respect to H_2 energy generation for a anaerobic bacterial granular fluidized bed bioreactor system (*AFBR*) with degassed effluent recycling (Ngoma *et al* 2011) can be estimated from the following energy balance relationship (Obazu *et al* 2012).

$$W_{net} = \left[B_{H_2} - P_{ir} - P_{er} - Q_i - R_e - H - \lambda E - Q_{H2O} - Q_{H_2} - Q_{CO_2} - W_{hp} + Q_{hp} + Q_{eo}\right]h = kWh\ (work) \quad (5.1)$$

$$W_{net} = \left[B_{H_2} - P_{ir} - P_{er} - Q_i - R_e - H - \lambda E - Q_{H2O} - Q_{H_2} - Q_{CO_2} - W_{hp} + Q_{hp} + Q_{eo}\right] = W\ (power)$$

where, W_{net} is the net work or net power produced by the bioreactor system; B_{H_2} is the power output from the bioreactor system in terms of total H_2 gas flux from the effluent gas disengager (L H_2/h); P_{ir} represents the electrical power required for pumping the nutrient influent through the fluidized granular bed; P_{er} represents the power required for recycling the degassed effluent through the fluidized granular bed; Q_i is electrical power required for increasing the temperature of the nutrient influent from its initial ambient

temperature (T_a) to the bioreactor's operational thermophilic temperature (T_b); R_e is the radiant energy emission flux from the bioreactor and gas-disengager surfaces; H is the free and forced convective or sensible heat flux from the bioreactor and gas-disengager surfaces; $\lambda E$is the latent heat flux from the effluent gas disengager ; Q_{H2O}, Q_{H_2} and Q_{CO_2}represents the quantity of heat absorbed by H_2O vapour, H_2 and CO_2 within the effluent gas disengager and lost as waste heat from the effluent gas-engager; W_{hp}represents the electrical power required for the operation of the heat-pump; Q_{hp}represents the heat energy delivered from the heat-pump; Q_{eo}heat energy recovered from the effluent overflow lost from the effluent gas disengager; $h$represents hours to convert power in W to energy in kWh.

If W_{net} is negative for H_2 generation for a *AFGB*, or for any other system, then the process would be energetically unviable. However, effective heat insulation and efficient heat recovery from the gas stream and from the effluent overflow stream would result in the performance of positive work by a thermophilicH_2 generating system (Obazu *et al*., 2012). With effective insulation the bioreactor system would be in thermal equilibrium with the surroundings and energy loses with respect to *R* and *H* would be zero. In addition, latent energy losses from the can be recovered through vapour condensation in the gas compression process. Heat recovery or heat recycling can be achieved through a continuous heat-pump process that involves compressing the heated gas flux from the effluent gas disengage (Obazu *et al*., 2012). The waste heat (Q_{waste}) flow into the heat-pump involves the uptake by the heat-pump of a gaseous working fluid (H_2O, H_2 and CO_2) expelled from the effluent gas disengager

Additional heat energy can also be recovered from the heated effluent overflow lost from the effluent gas disengager and recycling through a heat exchanger. The heat exchanger can be either used for heating up the nutrients stored in the nutrient supply reservoir or used in counter-current (tube-shell) configuration with regard to the influent feed line. The quantity of heat recovered (Q_{eo}) from the effluent overflow can be estimated as follows (Obazu *et al* 2012):

$$Q_{eo} = Q_i - \lambda E - Q_{H2O} - Q_{H_2} - Q_{CO_2} \quad (5.2).$$

In the bioreactor energy balance equation (equation 5.1) the major energy losses incurred with respect to the thermophilic generation of H_2 are from the following three sources: (1) the electrical power required (Q_i) for increasing the temperature of the nutrient influent; (2) radiant energy fluxes (R_e) from the surfaces of the bioreactor system; (3) free and forced convective or sensible heat fluxes (H) from the surfaces of the bioreactor system. Heat recovery from the effluent overflow (equation 5.2) and insulation of the bioreactor system will increase the overall energy balance efficiency of a thermophilic H_2 generating system.

The second important process goal involves exceeding the minimum economically acceptable volumetric supply rate of H_2 necessary for driving electricity generation from a 5 kW fuel cell. It has been estimated that the operation of a 5 kW fuel cell for electricity generation would require an H_2 supply rate of 2900 L H_2 /h (Levin.*et al*., 2004). In terms of volumetric hydrogen productivity (*HP*) this would be equivalent to 2.9 L H_2/(L.h) or 120 mmol H_2 /(L.h). Volumetric hydrogen productivities ranging from 7.3 L H_2/(L.h) to

14.8 L H_2/(L.h) have been achieved for mesophilic and thermophilic anaerobic fluidized bacterial granular bed bioreactors (Lee. *et al*., 2004; Lee.*et al*., 2006; Ngoma.*et al*., 2011).

In this study we evaluate whether this process goals can be met with regard to an *AFGB* system.

5.2. Materials and Methods

5.2.1. Medium

An Endo formulation (Endo.*et al*., 1982; Thompson.*et al*., 2006) was used as the nutrient medium for inoculum preparation and for the bioreactor experiments. The medium contained 17.8 g sucrose/L and the following mineral salts (g. L^{-1}) : NH_4HCO_3 6.72, $CaCl_2$ 0.2, K_2HPO_4 0.699, $NaHCO_3$ 3.36, $MgCl_2.6H_2O$ 0.015, $FeSO_4.7H_2O$ 0.0225, $CuSO_4.5H_2O$ 0.005, and $CoCl_2.H_2O$ 1.24 x 10^{-4}g.

5.2.2. Inoculum preparation

An undefined extreme thermophilic anaerobic bacterial consortium was derived from a mixture of sewage sludge and fresh cow dung. Sewage sludge was obtained from the overflow outlet of a mesophilic anaerobic digester at the Olifantsvlei wastewater treatment works (Johannesburg). Fresh cow dung was obtained from grass fed dairy cows at the Animal and Dairy Research Institute (Irene), Gauteng. Sewage and dung samples were incubated in Endo medium (50% v/v) at 90 ºC for 2 hours. After the heat treatment the pH of the samples were reduced to pH 2.0 with 0.1 N HCl. Inoculum samples were kept at this pH in sealed airtight Schott bottles for 12 h at room temperature and then

readjusted to pH 7.0 by mixing with Endo medium (50% v/v). The two inoculum preparations, sewage (1 L) and dung (1 L) were then applied to the bioreactor.

5.2.3. Bioreactor design and set-up

The bioreactor system consisted of the following 4 components: an influent and recycled effluent inlet manifold or diffuser, tubular bioreactor, a liquid-solid separator or sedimentation column connected to the top end of the tubular bioreactor and a tubular gas-disengager (Obazu *et al* 2012). Clear Perspex hollow tube was used for the construction of the tubular bioreactor (internal diameter (ID): 80 mm; height (H): 1000). The working volume for the tubular bioreactor's fluidized bacterial granular bed was 5 L. Volumetric hydrogen productivity was expressed in term of this volume rather than the total working volume of the bioreactor system. A 11.6 L liquid-solid separator was connected to the top end of the tubular bioreactor for solid-liquid separation to prevent the washout of the granules from bioreactor, especially at high effluent recycle rates. The solid - liquid separator consisted of two parts a 5.3 L component (ID: 150 mm and H: 300 mm) and a 6.3 L component (ID: 200 mm and H: 200 mm). At the base of the bioreactor the clear Perspex cylinder was connected to a conical shaped diffuser (ID: 80 mm and H: 150 mm) made from PVC which functioned as the primary inlet for the effluent recycle stream. A stainless steel sieve (32 mesh) was fixed over the inlet of the diffuser. Above the stainless steel sieve the conical diffuse was filled with a 100 mm layer of 5 mm glass beads. Positioned at the upper end of the diffuser were 4 inlet ports (ID 5 mm) with each inlet arranged at 90^{o} with respect to the two other inlets on each side. Nutrient medium (influent stream) was supplied directly into the upper glass bead layer via the 4 inlet

ports. The effluent overflow from solid-liquid separator was decanted into a gas-disengager which consisted of a gas collection cylinder (H: 200 mm and ID: 150 mm) connected to a gas-disengager cylinder (H: 600 mm and ID: 60 mm). The gas-disengager had two effluent outlets, one at the bottom that was connected to a variable Boyser® Bonfiglioli AMP-16 peristaltic pump (0.37 kW) which was used to recycle de-gassed effluent into the bioreactor via the diffuser. For effluent recycling the pump was set between 15 rpm and 50 rpm which gave a volumetric pumping rate ranging from 1.3 L/min to 3.5 L/min. The second effluent outlet drained the excess effluent overflow from the gas-disengager. The gas-disengager gas-outlet port was connected to a gas meter (Ritter drum-type gas meter TG 05/3). All Ritter drum gas meter measurements were carried out 25^{o}C. The liquid-gas separator or gas-disengager had a working volume of 1.54 L and the total fluid occupied volume of the interconnecting piping as 1.9 L. Total fluid containing volume of the bioreactor system (bioreactor bed, solid-liquid separator, gas-disengager, diffuser, and piping) was 20.0 L. Bioreactor and gas-disengager temperatures were maintained at the two operational temperatures, 60 ºC and 70 ºC, by circulating heated water from a heated water bath through the bioreactor and gas-disengager water jackets. A Watson-Mallow (model 520U) peristaltic pump (Falmouth, UK) was used to pump the Endo nutrient into the bioreactor.

5.2.4. Bacterial granule induction

On top of the glass bead bed a 100 mm bed of cylindrical activated carbon (*CAC*) particles (diameter = 2.5 mm and length = 5.0 mm) was used to facilitate the induction of

bacterial granulation in the bioreactor (Lee *et al*., 2004). Prior to its use, the *CAC* was first washed with distilled water to remove all the suspended fine particles and then sterilized by autoclaving for 20 minutes. Concentrated (3x) Endo medium (18.0 L) and seed inoculum (2.0 L) was added to the bioreactor system. Following inoculation the bioreactor was operated on a batch effluent-recycle mode for 48 h at 70^{o}C to acclimatize the bacteria and allow for their attachment to the *CAC*. After this acclimatization period the bioreactor operation was switched to continuous – effluent recycle mode with an initial hydraulic retention time (*HRT*) of 8 h, supplying Endo medium at its normal concentration. The *HRT* was then gradually decreased over 2 day intervals by increasing the nutrient medium supply rate. As the *HRT* was decreased from 8 to 4 h the growth and development of bacterial biofilm on the *CAC* particles became visible. With further decreases in the *HRT* below 4 h the biofilm growth increased and bacterial granules began to form and accumulate at the surface of the expanded *CAC* bed. Once granule formation had been initiated, further reductions in the *HRT* to between 2 and 1.6 h resulted in the rapid growth and expansion of the granular bed. Granule induction, initial growth and initial development was carried out at 70^{o}C.

5.2.5. Effluent recycle rate and effluent gas disengagement

The effluent discharged from the bioreactor was passed through a gas-disengager before being recycled back into the bioreactor (Ngoma *et al*., 2011). Effluent discharge force into the gas-disengager was dependent on the effluent recycle rate. High rates of effluent recycling between the bioreactor and the gas- disengage generated a high degree of fluid turbulence and cavitation within the gas disengager tube. This vigorous mixing process

within the gas-disengager facilitated the release of undissolved H_2 from the effluent through bubble production. Efficient removal of undissolved or non-solubilized H_2 trapped in the effluent phase by gas disengagement was expected to increase the overall biohydrogen production efficiency of the bioreactor system (Ngoma *et al*., 2011).

5.2.6. Analytical techniques

Gas chromatography was used to analyze% gas composition (H_2, CO_2 and CH_4). A Clarus 500 GC PerkinElmer equipped with a thermal conductivity detector was used. The temperatures of injector, detector and column (PerkinElmer Elite Q Plot capillary column 30 m x 32 mm) were kept at 250 °C, 200 °C and 45°C , respectively. Argon was used as the carrier gas at a flow rate of 2.0 ml min^{-1}. Sample gas injection volume was 40µl. The following formula (equation .5.3) was used for converting total bioreactor gas flux (L/h) to mmol H_2/h ,

$$\frac{\Delta H_2}{\Delta t} = \frac{P\left[\left(\%H^{GC}\right)\frac{\Delta V}{\Delta t}\right]}{RT} \qquad (5.3).$$

Where, $\Delta H_2/\Delta t$ = mmol H_2 /h; P = atmospheric pressure (85 kPa); ($\%H_2^{GC}$) = percentage hydrogen content from GC measurements; $\Delta V/\Delta t$ = L/h of total gas production from the gas meter measurements; R is the gas constant (8.314 J/(K.mol)); T = 298.15 K (the temperature at which the gas flow from the bioreactors were monitored).

The concentration of sucrose in the bioreactor influent and effluent streams was determined using the sucrose-resorcinol method (Kerr *et al*., 1984).

5.3. Result and Discussion

5.3.1. Effect of temperature on H_2 productivity

Nutrient supply rate or influent rate was 225 ml/min with a mean sucrose consumption rate of 91 ± 0.07% sucrose over all temperatures. Percentage hydrogen increased with increasing temperature, increasing from 46% at 55^{o}C to 71% at 70^{o}C (Figure 5.1). Similarly, total H_2 also increase with increasing, increasing from 21.5 L/h at 55^{o}C to 36.6 L/h at 70^{o}C. Volumetric H_2 production rates increased with temperature as follows: 147.64 mmol/L/h (55 °C), 194.22 mmol/L/h (60 °C) , 237.02 mmol/L/h (65 °C), 251.25 mmol/L/h 70 °C. (Figures 5.1 and 5.2) respectively. Hydrogen yield followed the same trend as % H_2 and total H_2 production, by increasing in response to increasing temperature (Figure 5.2).

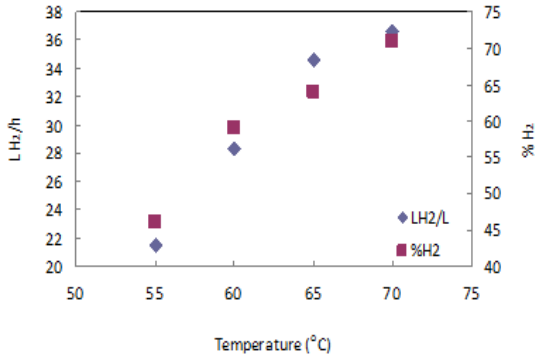


Fig 5.1

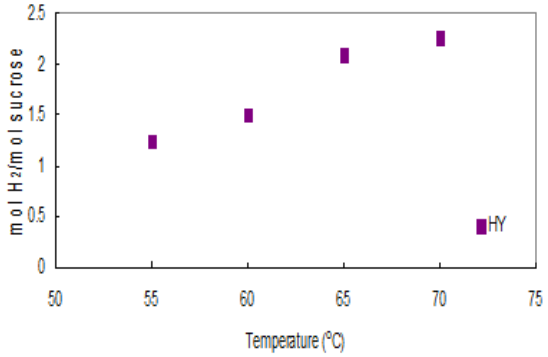


Fig 5.2

Figure 5.1: Shows volumetric H_2 production rates with temperature increase and **Figure 5.2:** Describe hydrogen yield increase in response to temperature increase correspondingly

5.3.2 Net energy output.

Table 5.1 gives the net hydrogen energy output at the different temperatures. Net hydrogen energy hydrogen energy output is defined as follows:

$$E_{output} = B_{H2} + Q_{rec} - E_{input}$$

Where E_{input} is the total energy input used for the bioreactor operation and Q_{rec} is the heat recovered. The estimated bioreactor energy production efficiencies for the bioreactor

system were ranged between 49% to 78%. The capacity for the bioreactor to generate positive work will be dependent on the efficiency heat energy recovery.

Table 5.1: Show the net H_2 energy output at the different temperatures

T	Influ-ent rate	Effluent recycle rate	H_2 product ion	H_2 energy	Influent pump	Effluent pump	Influent Heating	Latent heat loss	H_2O vapour heat flux	H_2 heat flux	CO_2 heat flux	Total energy input	Heat recovery	Net energy output
T_b	F_{ir}	F_{er}	G_H	B_{H2}	P_{ir}	P_{er}	Q_i	λE	Q_{H2O}	Q_{H2}	Q_{CO2}	E_{input}	Q_{rec}	E_{output}
^oC	L/h	L/min	L H_2/h	W	W	W	W	W	W	W	W	W	W	W
55	13.5	3.5	21.5	75.95	-1.14	-31.79	-471.38	-2.44	-0.06	-0.18	-0.11	-507.1	468.59	37.44
60	13.5	3.5	28.3	99.91	-1.14	-31.79	-549.94	-3.10	-0.08	-0.21	-0.13	-586.39	546.42	59.94
65	13.5	3.5	34.6	121.92	-1.14	-31.79	-628.50	3.89	-0.12	-0.24	-0.15	-658.05	631.88	95.75
70	13.5	3.5	36.6	129.24	-1.14	-31.79	-707.06	-4.83	-0.17	-0.27	-0.17	-745.43	701.62	85.43

$$E_{output} = B_{H2} + Q_{rec} - E_{input}$$

5.3.3 Efficiency of electricity generation capacity

Efficiency of electricity generation capacity can be defined by the power out per m^3 in terms of H_2 required for the operation of a 5 kW cell. The *HPs* for a scaled up version of the bioreactor would be: 4300 L H_2/m^3 (55^{o}C), 5660 L H_2/m^3 (60^{o}C), 6920 L H_2/m^3 (65^{o}C), 7320 L H_2/m^3 (70^{o}C). The values are 1.48, 1.95, 3.39, and 2.59 greater than the processed 2900 L H_2/m^3 required to operate a 5 kW fuel cell (Levin *et al* 2004).

5.4 Conclusion

The study showed that a positive net energy balance at thermophilic temperatures and high effluent recycle rates were attainable and the volumetric hydrogen productivities were sufficient to drive a 5 kW fuel cell when scale-up to 1 m^3. However, an increase in

the *HYs* above the 75% value (3 mol H_2/mol glucose) of so-called Thauer limit was not attained. In chapter six I report on the bioreactor conditions that make this possible.

CHAPTER SIX

INTERRELATIONSHIPS BETWEEN BIOREACTOR VOLUME, EFFLUENT RECYCLE RATE, TEMPERATURE, pH, %H_2, HYDROGEN PRODUCTIVITY AND HYDROGEN YIELD WITH UNDEFINED BACTERIAL CULTURES.

6.1 Introduction

To date, the thermodynamic or substrate conversion efficiency of dark anaerobic biohydrogen production for all anaerobic fluidized granular bed bioreactors (*AFGBs*) has not exceeded 3 mol H_2 /mol glucose (Ngoma *et al*., 2011). The reason for this is that the experimental conditions under which high hydrogen productivities (*HPs*) have been achieved do not favour the simultaneous achievement of hydrogen yields (*HYs*) greater than 2 mol H_2/mol glucose (Ngoma *et al*., 2011; Lee *et al*., 2004, 2006; Zhang *et al*., 2008; O-Thong *et al*., 2008 and Thompson *et al*., 2008). Achievement of *HYs* greater than 2.0 mol H_2/mol glucose in bioreactor experiments have been dependent on the following operation conditions: monocultures, thermophilic temperatures, low substrate loading rates, low dilution rates, H_2 gas stripping by sparging with N_2, maintenance of low H_2 partial pressures (< 100 Pa) and low bacterial biomass densities (De Vrije *et al*., 2007; Zeiden and van Niel, 2010). Bioreactor experiments that achieved high *HPs* have depended on the following operational conditions: undefined multispecies bacterial consortia, high substrate loading rates, high H_2 partial pressures, high dilution rates, and high bacterial biomass densities glucose (Ngoma *et al*., 2011; Lee *et al*., 2004, 2006; Zhang *et al*., 2008; O-Thong *et al*., 2008 and Thompson *et al*., 2008).

Biohydrogen production by the various *AFGB* systems represents significant technological advance glucose (Ngoma *et al*., 2011; Lee *et al*., 2006; Zhang *et al*., 2008). Further development of the *AFGB* system will depend on improving H_2 liquid-to-gas mass transfer of H_2 . Factors which constrain H_2 liquid-to-gas mass transfer will limit the simultaneous achievement high *HPs* and high *HYs*. Liquid-to-gas phase mass balance dynamics for H_2 assumes an equilibrium distribution of H_2 based on Henry's law. However, the low solubility and low mass transfer coefficients of gases like H_2 can delay the attainment of thermodynamic equilibrium between the different phases of the *AFGB* system. For example, gaseous H_2 fluxes from the *AFGB* system involves H_2 mass transfers between three different material phases in which also happen to be in different states of motion. In the *AFGB* system we have the co-existence of a quasi-static solid phase, a mobile bulk fluid or liquid phase and a mobile gaseous phase. The quasi-static solid phase consists of the fluidized bacterial granular bed which functions as the H_2 generating *biocatalyst*. The mobile liquid phase consists of the nutrient influent and recycled degassed effluent. The mobile gaseous phase consists of gas bubbles generated through the process of cavitation or bubble nucleation within the bioreactor. The latter two mobile phases also shift the anaerobic oxidation processes away from the thermodynamic equilibrium state by removing H_2 from the quasi-static solid phase or from the biocatalyst surface. In a majority of the *AFGB* systems the bacterial granules consists of a multispecies microbial consortium (Ngoma *et al*., 2011; Lee *et al*., 2006; Zhang *et al*., 2008).

Currently, the low space/time yields (*STYs*) per unit volume has discouraged the commercialization of biohydrogen production (Nath and Das, 2011). Increases in the *STY*

for biohydrogen generation require a simultaneous increase in both *HP* and *HY*. In the *AFGB* system the combination of high nutrient influent rates and high rates of degassed effluent recycling has been observed to result in *HPs* as high as 14.8 L H_2/L/h with an *HY* equal to 2.2 mol H_2 /mol glucose (Ngoma *et al*., 2011). Hypothetically, by decreasing the total of liquid volume of the *AFGB* system (V) relative to the degassed effluent recycle rate (F_{er}) a simultaneous increase in both *HP* and *HY* can be achieved. The total volume (V) of an *AFGB* system consists of the sum of the working bioreactor volume (*B*), the volume of the solid-liquid separator column (*S*) above the actual bioreactor, the volume of the gas-disengager (*G*) and finally the volume of the piping (*P*). This hypothesis predict that for some critical value X, where $X = V/F_{er}$, *HP* will be some factor greater than 120 mmol H_2/L/h, and *HY* will be equal to or greater than 3.0 mol H_2/mol glucose. It should be noted that irrespective of the *HY* attained for any bioreactor system, electricity generation by a 5 kW fuel cell would require an H_2 supply rate not less than 2900 L H_2 /h (Levin *et al*., 2004). This would be equivalent to a volumetric hydrogen production rate of 2.9 L H_2/L/h or 120 mmol H_2 /L/h. Once this level of volumetric hydrogen productivity has been attained, or preferably exceeded, the next bioprocess optimization objective would then be to increase the *HY* to 3 mol H_2/ mol glucose, or better still, to a value exceeding this. A commercially viable STYs per unit volume should aim at achieving the *HPs* greater than 120 mmol H_2 /L/h and *HYs* greater than 3 mol H_2/ mol glucose. While information regarding the *HPs* and *HYs* attainable for various *AFGB* systems have been well documented (Ngoma *et al*., 2011), to date no comprehensive energy balance analysis of any *AFGB* system has been undertaken. In this study an

energy balance model for a thermophilic *AFGB* system was also developed in order to ascertain whether such a system could also achieve a net positive energy balance.

6.2. Materials and Methods

6.2.1.Medium

An Endo formulation (Endo *et al*., 1982 and Thompson *et al*., 2006) was used as the nutrient medium for inoculum preparation and for the bioreactor experiments. The medium contained 17.8 g sucrose/L and the following mineral salts (g. L^{-1}) : NH_4HCO_3 6.72, $CaCl_2$ 0.2, K_2HPO_4 0.699, $NaHCO_3$ 3.36, $MgCl_2.6H_2O$ 0.015, $FeSO_4.7H_2O$ 0.0225, $CuSO_4.5H_2O$ 0.005, and $CoCl_2.H_2O$ 1.24 x 10^{-4}g.

6.2.2. Inoculum preparation

An undefined bacterial consortium that had the capacity to generate hydrogen under temperatures ranging from 45^{o}C to 70 ^{o}C was derived from a mixture of sewage sludge and fresh cow dung. Sewage sludge was obtained from the overflow outlet of a mesophilic anaerobic digester at the Olifantsvlei wastewater treatment works (Johannesburg). Fresh cow dung was obtained from grass fed dairy cows at the Animal and Dairy Research Institute (Irene), Gauteng. Sewage and dung samples were incubated in Endo medium (50% v/v) at 90 ºC for 2 hours. After the heat treatment the pH of the samples were reduced to pH 2.0 with 0.1 N HCl. Inoculum samples were kept at this pH in sealed airtight Schott bottles for 12 h at room temperature and then readjusted to pH 7.0 by mixing with Endo medium (50% v/v). The two inoculum preparations, sewage (1 L) and dung (1 L) were then applied to the bioreactor.

6.2.3. Bioreactor design and set-up

The overall generic design of the bioreactor systems used has been in Figure 3.1(chapter three) and Table 6.1. Basically the bioreactor consists of a number of components: an influent and recycled effluent inlet manifold or diffuser, tubular bioreactor compartment (*B*) that housed the bacterial granular bed, a liquid-solid separator or sedimentation column (*S*) connected to the top end of the tubular bioreactor which prevents washout of the bacterial granular bed and a tubular gas-disengager (*G*) into which the hydrogen saturated effluent is discharged (Figure 3.1). The dimensions of *B*, *S*, and *G* (diameter and length) and their corresponding volumes (L) for the 5 different bioreactor configurations (R1, R2, R3, R4, R5) are given in Table 6.1. In order to reduce the total bioreactor system volume (*V*) the liquid-solid separator was removed from R5. Clear Perspex hollow tube was used for the construction of *B*, *S*, and *G*. The effluent gas-disengager consisted of a gas collection cylinder (H: 200 mm and ID: 150 mm) connected to an effluent gas-disengager cylinder (H: 545 mm and ID: 60 mm). The effluent gas-disengager had two effluent outlets, one at the bottom that was connected to a variable Boyser® Bonfiglioli AMP-16 peristaltic pump (0.37 kW) which was used to recycle de-gassed effluent into the bioreactor via the diffuser. For effluent recycling the pump was set between 15 rpm and 50 rpm which gave a volumetric pumping rate ranging from 1.3 L/min to 3.5 L/min. The maximum pump discharge pressure at the bioreactor inlet was 4 bar. The second effluent outlet drained the excess effluent overflow from the gas-disengager. The gas-disengager gas-outlet port was connected to a gas meter (Ritter drum-type gas meter TG 05/3). All Ritter drum gas meter measurements were carried out 25 ^{o}C. The liquid-gas separator or effluent gas-disengager had a working volume of 1.54 L.

Table 6.1: The dimensions of the different components of the 5 different *AFGB* systems. See Fig.3.1 for generic diagram of *AFGB* system.

Bioreactor system	Bioreactor dimensions *B*			Separator dimensions *S*			Gas disengager dimensions *G*			Pipe volume *P*	Total volume *V=B+S+G+P*
	Radius (mm)	Length (mm)	Volume (L)	Radius (mm)	Length (mm)	Volume (L)	Radius (mm)	Length (mm)	Volume (L)	(L)	(L)
R1	40	1000	5.0	75 100	200 300	13.0	30	545	1.54	1.90	19.1
R1	40	1000	5.0	75 100	200 300	13.0	30	545	1.54	1.90	19.1
R2	40	1000	5.0	100	320	10.0	30	545	1.54	1.46	18.0
R3	40	1000	5.0	100	160	5.0	30	545	1.54	1.08	12.6
R3	40	1000	5.0	100	160	5.0	30	545	1.54	1.08	12.6
R4	40	650	3.27	100	147	4.62	30	545	1.54	1.08	10.5
R5	40	650	3.27	No separator		0	30	545	1.54	0.93	5.74
R5	40	650	3.27			0	30	545	1.54	0.93	5.74
R5	40	650	3.27			0	30	545	1.54	0.93	5.74
R5	40	650	3.27			0	30	545	1.54	0.93	5.74

At the base of the bioreactor (*B*) the clear Perspex cylinder was connected to a conical shaped diffuser (ID: 80 mm and H: 150 mm) made from PVC which functioned as the primary inlet for the effluent recycle stream. A stainless steel sieve (32 mesh) was fixed over the inlet of the diffuser. Above the stainless steel sieve the conical diffuse was filled with a 100 mm layer of 5 mm glass beads. Positioned at the upper end of the diffuser were 4 inlet ports (ID 5 mm) with each inlet arranged at 90^{o}C with respect to the two other inlets on each side. Nutrient medium (influent stream) was supplied directly into the upper glass bead layer via the 4 inlet ports. Total fluid containing volume of the different bioreactor systems (bioreactor bed, solid-liquid separator, gas-disengager, diffuser, and piping) is shown in Table 6.1. Bioreactor and gas-disengager temperatures were maintained at the various operational temperatures (T_b) by circulating heated water from a heated water bath through the bioreactor and gas-disengager water jackets. A

Watson-Mallow (model 520U) variable peristaltic pump (Falmouth, UK) with a power rating of 115/230V was used to pump the Endo nutrient into the bioreactor. The discharge pressure of the influent pump was 2 bars.

6.2.4. Bacterial granule induction

On top of the glass bead bed a 100 mm bed of cylindrical activated carbon (*CAC*) particles (diameter = 2.5 mm and length = 5.0 mm) was used to facilitate the induction of bacterial granulation in the bioreactor (Lee *et al*., 2004). Prior to its use, the *CAC* was first washed with distilled water to remove all the suspended fine particles and then sterilized by autoclaving for 20 minutes. Concentrated (3x) Endo medium and seed inoculum (2.0 L) was added to the bioreactor system. Following inoculation the bioreactor was operated on a batch effluent-recycle mode for 48 h to acclimatize the bacteria to thermophilic temperatures (60^{o}C, 65^{o}C and 70^{o}C) and allow for their attachment to the *CAC*. After this acclimatization period the bioreactor operation was switched to continuous – effluent recycle mode with an initial hydraulic retention time (*HRT*) of 8 h, supplying Endo medium at its normal concentration. The *HRT* was then gradually decreased over 2 day intervals by increasing the nutrient medium supply rate. As the *HRT* was decreased from 8 to 4 h the growth and development of bacterial biofilm on the *CAC* particles became visible. With further decreases in the *HRT* below 4 h the biofilm growth increased and bacterial granules began to form and accumulate at the surface of the expanded *CAC* bed. Once granule formation had been initiated, further reductions in the *HRT* to between 2 and 1.6 h resulted in the rapid growth and expansion

of the granular bed. Granule induction, initial growth and initial development were carried out at thermophilic temperatures.

6.2.5. Analytical techniques

Gas chromatography was used to analyze % gas composition (H_2, CO_2 and CH_4). A Clarus 500 GC PerkinElmer equipped with a thermal conductivity detector was used. The temperatures of injector, detector and column (PerkinElmer Elite Q Plot capillary column 30 m x 32 mm) were kept at 250 °C, 200 °C and 45°C , respectively. Argon was used as the carrier gas at a flow rate of 2.0 ml min^{-1}. Sample gas injection volume was 40µl. The following formula (equation 6.1) was used for converting total bioreactor gas flux (L/h) to mmol H_2/h ,

$$\frac{\Delta H_2}{\Delta t} = \frac{60 P_a [\%H_2^{GC} G_T]}{R T_a} \qquad 6.1$$

Where, $\Delta H_2/\Delta t$ = mmol H_2 /h; P_a = atmospheric pressure (85 kPa); $\%H_2^{GC}$ = percentage hydrogen content from GC measurements; G_T (L/min) represents the total gas production rate from the gas meter measurements; R is the gas constant (8.314 J/K/mol); T_a= 298.15 K (the temperature at which the gas flow from the gas meter were monitored).

The concentration of sucrose in the bioreactor influent and effluent streams was determined using the sucrose-resorcinol method (Kerr *et al*., 1984).

6.2.6. Experimental design and hypothesis testing

Different bioreactor configurations were used to test the hypothesis that a decrease in value of the ratio V/F_{er} (min) will result in simultaneous increase in both *HP* and *HY*, where *V* in L represents the total *AFGB* system volume ($V = B + S + G + P$, as defined in Table 6.1) and F_{er} represents the degassed effluent recycle rate in L/min. Different bioreactor configurations were constructed by reducing the volumes of the tubular bioreactor, the solid-liquid separator and the interconnecting tubing (Table 6.1). A total of five different bioreactor configurations (R1, R2, R3, R4, R5) were used to generate 5 different V/F_{er} values (5.46 min, 5.14 min, 3.61 min, 3.28 min, 2.86 min, 1.79 min, see Table 6.2), where the V/F_{er} represents the time taken in minutes to pass the total bioreactor system fluid volume through the effluent gas disengager.

Table 6.2: The combined influence of the following factors: temperature (T_b), degassed effluent recycle rate (F_{er}), total bacterial granule biomass, bioreactor system volume on volumetric hydrogen productivity (*HP*), hydrogen yield (*HY*), and % H_2 content. The total granule dry mass corresponds to the bioreactor working volume (*B*). *V* = total bioreactor system volume = $B + S+G+P$.

R	T	pH	Influent rate	Effluent recycle rate	Total Volume: effluent recycle rate ratio	Bioreactor volume: effluent recycle rate ratio	Total granule dry mass	Bacterial granule biomass density	% H_2	*HP*	*HY*
	(°C)		(L/h)	(L/min)	(min)	(min)	(g)	(g/L)		(mmol H_2/L/h)	(mol H_2/mol glucose)
					V/F_{er}	*B/Fer*					
R1	60	7.2	13.5	3.5	5.46	1.43	135.7	27.1	69	269.8	1.15
R1	70	7.2	13.5	3.5	5.46	1.43	135.7	27.1	77	342.4	1.34
R2	65	7.1	9.6	3.5	5.14	1.43	181.0	36.2	57	211.1	1.21
R3	45	5.4	13.5	3.5	3.61	1.43	45.2	9.0	48	142.2	1.24
R3	70	5.5	13.5	3.5	3.61	1.43	52.8	10.6	82	420.1	1.84
R4	45	6.0	4.5	3.2	3.28	1.02	40.7	12.5	42	175.5	1.61
R4	65	7.1	4.5	3.5	2.86	1.43	40.7	12.5	53	240.3	2.84
R5	45	5.6	4.5	3.2	1.79	1.02	42.2	12.9	40	113.1	2.21
R5	60	6.0	4.5	3.2	1.79	1.02	42.2	12.9	55	190.2	3.00
R5	65	7.2	4.5	3.2	1.79	1.02	42.2	12.9	60	223.8	3.34
R5	70	7.8	4.5	3.2	1.79	1.02	42.2	12.9	62	231.3	3.55

6.2.7. Theory for H_2 gas-disengagement from the effluent

The *AFGB* bioprocess for H_2 production involves the interaction of three different phases: a quasi-static solid phase, a mobile bulk fluid or liquid phase and a mobile gaseous phase. The quasi-static solid phase consists of the fluidized bacterial granules. The mobile liquid phase consists of the two fluid fluxes, the nutrient influent flux (F_{ir}) and the recycled degassed effluent flux (F_{er}). The mobile gaseous phase consists of gas bubbles generated through the combined processes of cavitation, bubble nucleation and bubble amalgamation. The bacterial granules are comprised of a multispecies bacterial consortium.

The effluent discharged from the bioreactor (*B*) was passed through a gas-disengager (*G*) before being recycled back into the bioreactor (Ngoma *et al*., 2011). Effluent discharge force into the gas-disengager was dependent on the effluent recycle rate. High rates of effluent recycling between the bioreactor and the gas- disengage generated a high degree of fluid turbulence and cavitation within the gas disengager tube. This vigorous mixing process within the gas-disengager facilitated the release of undissolved H_2 from the effluent through bubble production. Efficient removal of undissolved or non-solubilized H_2 trapped in the effluent phase by gas disengagement was expected to increase the overall biohydrogen production efficiency of the bioreactor system (Ngoma *et al*., 2011).

At high *HPs* the low solubility and low mass transfer coefficient values for dissolved H_2 results in the buildup of super-saturation levels of H_2 trapped within the liquid phase. The solubility of H_2 at 0 ^{o}C (101.3 kPa) is 0.00192 g/L. Its solubility only decreases slightly to between 0.0011 and 0.0012 g/L as the temperature increases to

between 60 ^{o}C and 70 ^{o}C. The super-saturated total concentration (H_2^L) consists of the combined concentrations of dissolved and non-dissolved H_2 trapped in the liquid phase. The super-saturated total concentration of H_2 in relation to the thermodynamic equilibrium concentration of H_2 , the volumetric productivity of hydrogen and the liquid phase volumetric mass transfer coefficient for H_2 can be defined as follows:

$$H_2^L = H_2^{L*}\left[\frac{HP}{K_H^T RT k_L a} - 1\right] \qquad 6.2,$$

where H_2^{L*} (mole H_2 /L) is the thermodynamic equilibrium concentration of H_2 dissolved in the liquid phase, HP(mole H_2/L/h) is the volumetric hydrogen productivity, R (8.314 Pa/mole/K) is the gas constant, K_H^T (mole/L/Pa) is Henry's constant, and $k_L a$ (h^{-1}) is the liquid phase volumetric mass transfer coefficient for H_2 in aqueous solutions, a (m^2/L) is the specific interfacial area per unit volume of liquid in the bioreactor. Under ideal conditions the thermodynamic equilibrium concentration of dissolved hydrogen is related to partial pressure by Henry's law as follows

$$P_H = K_H^L H_2^{L*} \qquad 6.3,$$

where, P_H is the partial pressure of hydrogen, K_H^L equals 1282.05 L/atm/mol

In equation 6.2 term k_L in the liquid phase volumetric mass transfer coefficient represents the film coefficient (Pauss *et al*., 1990). The values for both k_L and a depend on the combined effects of multiple factors such as liquid surface tension, fluid viscosity, effluent recycle rate, temperature, cavitation rate or bubble formation rate, upward

velocity of bubbles formed, bubble diameter, size of particles such as the granules dispersed in the liquid phase. The effluent recycle rate is an example of a power input factor. All of these factors will influence the two processes that bring about the removal of dissolved H_2 from the surface of granules: (1) diffusion and convection, and (2) transfer into gas bubbles (Figure 6.1). The rate of transfer of H_2 from the liquid phase to the gaseous phase with the *AFGB* system has been modified after Pauss *et al*., 1990 (Pauss *et al*., 1990),

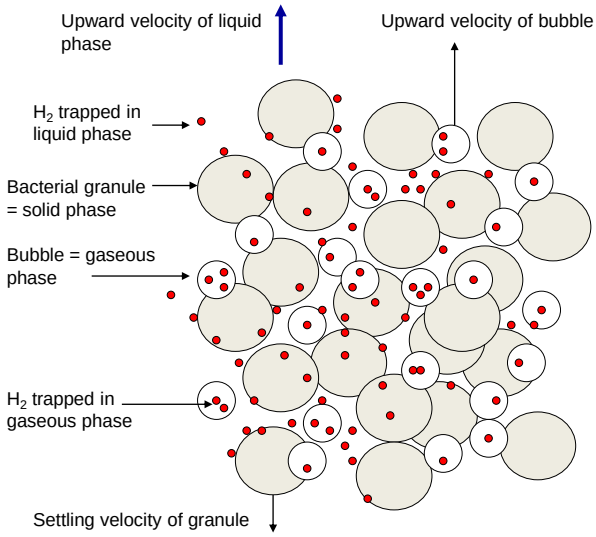


Figure 6.1: The partitioning of non-dissolved and soluble H_2 between the three different phases in the *AFGB* system: a quasi-static solid phase comprised of a fluidized bed of bacterial granule particles, a mobile bulk fluid or liquid phase and a mobile gaseous phase. The mobile liquid phase consists of the two fluid fluxes, the nutrient influent flux and the recycled degassed effluent flux. The mobile gaseous phase consists of gas bubbles filled with water vapour, H_2 and CO_2 .

$$\frac{d(H_2^L)_L}{dt} = D(H_2^{L*})_L - D(H_2^L)_L + \rho_B(SHP) - k_L a[(H_2^L)_L - (H_2^{L*})_L] \quad 6.4,$$

$$\frac{d(H_2^L)_G}{dt} = \frac{V}{V_G} k_L a[(H_2^L)_L - (H_2^{L*})_L] - \frac{G_{H_2}\rho_{H_2}}{V_G R T_b} \quad 6.5,$$

where, the term $(H_2^L)_L$ represents the concentration of super-saturated H_2 trapped in the liquid phase, the term $(H_2^L)_G$ represents the concentration of super-saturated H_2 transferred into and then trapped in the gaseous phase; $(H_2^{L*})_L$ represents the thermodynamic equilibrium concentration of dissolved H_2 re-entering the bioreactor via the recycled degassed effluent stream; $(H_2^L)_L$ also represents the concentration of super saturated H_2 trapped in the effluent stream discharged from the bioreactor into the effluent gas-disengager (Fig. 6.1); D is the dilution rate, $D = F_{sr}/V$ (min^{-1}) is the dilution rate; *SHP* (mol H_2/g/h) is the specific hydrogen productivity of the fluidized granular bed; V (L) also represents the total liquid phase volume of the bioreactor system; V_G (L) represents the total gaseous phase volume of the bioreactor; ρ_{H_2} (mol H_2 /L) is the density of the H_2 in the gaseous phase; G_{H_2} (L H_2/h) is the total H_2 gas flow rate from the gas-disengager; and T_b represents the bioreactor system temperature. Under steady state conditions the following equality obtains:

$$D(H_2^L)_L - D(H_2^{L*})_L + \rho_B(SHP) = \frac{G_{H_2}\rho_{H_2}}{VRT_b} \quad 6.6,$$

where, ρ_B bacterial granular biomass density. Rearrangement of equation 6.6 and substitution of F_{er} /*V* for *D* shows the relationship between the rate of total H_2 ($G_{H_2}\rho_{H_2}$) production and F_{er} , *V*, SHP and ρ_B

$$G_{H_2}\rho_{H_2} = F_{er}RT_b(H_2^L)_L - F_{er}RT_b(H_2^{L*})_L + VRT_b\rho_B(SHP) \qquad 6.7.$$

From equation 5.7 a relation between *HY* and the rate of effluent gas-disengagement can demonstrated:

$$HY = \frac{G_{H_2}\rho_{H_2}}{V\rho_B(SGC)} = \frac{F_{er}RT_b(H_2^L)_L - F_{er}RT_b(H_2^{L*})_L + VRT_b\rho_B(SHP)}{V\rho_B(SGC)} \qquad 6.8,$$

where SGC(mol glucose/g/h) represent the specific glucose consumption rate. Equation 6.8 predicts that if F_{er} and T_b are increased and $V$is decreased *HY* will increase for a given ρ_B. The term $F_{er}RT_b(H_2^L)_L - F_{er}RT_b(H_2^{L*})_L$ in equation 6.7 and 6.8 has been assumed to accurately represent effluent gas-disengagement.

6.2.8. Energy balance model for thermophilic biohydrogen production

Equation 6.8 shows that the magnitude of *HY* is directly dependent on the application of external work on the bioreactor system in the form of mechanical energy associated with F_{er} and in the form of heat energy for the maintenance of T_b. In this energy balance model the net work done by the thermophilic bioreactor system with respect to H_2 energy generation can be estimated from the following energy balance relationship (equation 6.9) :

$$W_{net} = [B_{H_2} - P_{ir} - P_{er} - Q_i - R_e - H - \lambda E - Q_{H2O} - Q_{H_2} - Q_{CO_2} - W_{hp} + Q_{hp} + Q_{eo}]h = kWh\ (work)$$

or

$$W_{net} = [B_{H_2} - P_{ir} - P_{er} - Q_i - R_e - H - \lambda E - Q_{H2O} - Q_{H_2} - Q_{CO_2} - W_{hp} + Q_{hp} + Q_{eo}] = W\ (power)$$

where, W_{net} is the net work or net power produced by the bioreactor system; B_{H_2} is the power output from the bioreactor system in terms of total H_2 gas flux from the effluent gas disengager (L H_2/h); P_{ir} represents the electrical power required for pumping the nutrient influent through the fluidized granular bed; P_{er} represents the power required for recycling the degassed effluent through the fluidized granular bed; Q_i is the electrical power required for increasing the temperature of the nutrient influent from its initial ambient temperature (T_a) to the bioreactor's operational thermophilic temperature (T_b); R_e is the radiant energy emission flux from the bioreactor and gas-disengager surfaces; H is the free and forced convective or sensible heat flux from the bioreactor and gas-disengager surfaces; λE is the latent heat flux from the effluent gas disengager ; Q_{H2O} , Q_{H_2} and Q_{CO_2} represents the quantity of heat absorbed by H_2O vapour, H_2 and CO_2 within the effluent gas disengager and lost as waste heat from the effluent gas-engager; W_{hp} represents the electrical power required for the operation of the heat-pump; Q_{hp} represents the heat energy delivered from the heat-pump; Q_{eo} heat energy recovered from the effluent overflow lost from the effluent gas disengager; h represents hours to convert power in W to energy in kWh. Hydrogen energy produced by the bioreactor system was estimated as follows:

$$B_{H_2} = \frac{(HP)B\rho_{EH_2}}{3600 \cdot 1000} \qquad 6.10,$$

where, HP is the volumetric hydrogen productivity in L H_2/L/h, B is the bioreactor working volume in L; ρ_{EH_2} is the volumetric hydrogen energy density (12.7 MJ/m^3).

Heating of the nutrient influent stream (Q_i) and recycling of the degassed effluent stream (P_{er}) represents the two largest energy requirements for the operation of the current thermophilic *AFGB* system. Energy required for nutrient influent heating can be defined as follows:

$$Q_i = C_{pw} F_{ir} [T_b - T_a] \qquad 6.11$$

where, C_{pw} is the specific heat of water (4.19 kJ/kg/^{o}C); F_{ir} is the influent or nutrient supply rate (L/s, assume L = kg); T_b is temperature (^{o}C) of the bioreactor's liquid phase; T_a is the temperature (^{o}C) of the liquid phase in the nutrient storage tank. Electrical power required for degassed effluent recycling can be estimated as follows:

$$P_{er} = \frac{\rho_w F_{er} g H_{er}}{\eta_p \eta_m 60 \cdot 1000} \qquad 6.12,$$

where, ρ_w (1000 kg/m^3) is the density of water; F_{er} (L/min) is the degassed effluent recycle rate (division by 60 and 1000 converts the rate to m^3/s); g (9.81 m/s^2) is the gravitational acceleration; H_{er} (m) the head rise for degassed effluent recycling pump; η_p (0.75) is the dimensionless pumping efficiency; η_m (0.90) is the dimensionless electrical motor efficiency. The head rise was estimated using Bernoulli's equation

$$H_{er} = \frac{P_{dp}^{er}}{\rho_w g} + \frac{V_{er}^2}{2g} + H_b \qquad 6.13,$$

where, P_{dp}^{er} (4 x10^5 N/m^2) is the discharge pressure of the degassed effluent recycle pump; V_{er} (m/s) is the linear degassed effluent flow velocity calculated from F_{er}; and H_b (m) is the actual height to which the fluid is pumped within the bioreactor system. Power requirements for influent supply are given by the following two equations:

$$P_{ir} = \frac{\rho_w F_{ir} g H_{ir}}{\eta_p \eta_m 3600 \cdot 1000} \qquad 6.14,$$

where, F_{ir} (L/h) is the influent flow rate and the influent supply head rise H_{ir} is defined as follows:

$$H_{ir} = \frac{P_{dp}^{ir}}{\rho_w g} + \frac{V_{ir}^2}{2g} + H_b \qquad 6.15,$$

where, P_{dp}^{ir} (2 x 10^5 N/m^2) is the pump's discharge pressure and V_{ir} (m/s) is the influent supply linear flow velocity.

The latent heat losses were computed for the water vapour losses (E, kg/s) from effluent gas disengager. The equilibrium saturated vapour pressure (e_s, kPa) within the effluent gas disengager was estimated using the following empirical relationship (Gray, 2003)

$$e_s = 0.611exp\left[\frac{17.5T_b}{T_b + 241}\right] \qquad 6.16.$$

Latent heat energy loss from the gas disengager was computed using the following relationship:

$$\lambda E = \frac{\lambda 18 e_s G_T}{60RT_b} \qquad 6.17,$$

where, λ is the latent heat of evaporation constant (2.42 MJ/kg, 25^{o}C) and G_T (L/s) is the total gas flux from the effluent gas disengager. The temperature depend value for λ was estimated using the following empirical relation (Gray, 2003):

$$\lambda = 2.501 - 0.00296T_b \qquad 6.18.$$

If W_{net} is negative for H_2 generation for the current thermophilic *AFGB* system then the process would be energetically unviable. However, effective heat insulation and efficient heat recovery from the gas stream and from the effluent overflow stream would result in the performance of positive work by a thermophilic *AFGB* system (Groenestijn *et al*., 2002). With effective insulation, the bioreactor system would be in thermal equilibrium with the surroundings and energy losses with respect to *R* and *H* would be zero. In addition, latent energy losses from the effluent gas disengager can be recovered through vapour condensation in the gas compression process. Heat recovery or heat recycling can be achieved through a continuous heat-pump process that involves compressing the heated gas flux from the effluent gas disengager van Groenestijn *et al*., 2002). The waste heat (Q_{waste}) flow into the heat-pump involves the uptake by the heat-pump of a gaseous working fluid expelled from the effluent gas disengager. The expelled

working fluid is composed of a mixture of water vapour, H_2 and CO_2 at an initial pressure and temperature of P_{in} and T_{in} , respectively. The waste heat released from the effluent gas disengager and taken up by the heat-pump can be defined as follows :

$$Q_{waste} = Q_{H2O} + Q_{H_2} + Q_{CO_2} \qquad 6.19,$$

where:

$$Q_{H2O} = C_{pH2O}\dot{m}_{H2O}\Delta T \qquad 6.20,$$

$$Q_{H_2} = C_{pH_2}\dot{m}_{H_2}\Delta T \qquad 6.21,$$

$$Q_{CO_2} = C_{pCO_2}\dot{m}_{CO_2}\Delta T \qquad 6.22,$$

and where, C_{pH2O} (1.84 kJ/kg/K, 25^{o}C), C_{pH_2}(14.267 kJ/kg/K, 25^{o}C) and C_{pCO_2}(0.85 kJ/kg/K, 25^{o}C) are the specific heat capacities for H_2O vapour, H_2 and CO_2 respectively; $\dot{m}_{H20}$, $\dot{m}_{H_2}$ and $\dot{m}_{CO_2}$ are the H_2O vapour, H_2 and CO_2 mass fluxes (kg/s) from the effluent gas disengager, respectively. For ease of calculation $\Delta T = T_b - T_a$.

For the electrical power required for the operation the heat-pump system can be estimated as follows (http://charming.awardspace.com/heat_pump/heat_pump_html)

$$W_{hp} = \frac{P_{in}\frac{G_T}{60}}{T_{in}} \cdot \frac{1}{k-1}[T_{out} - T_{in}]\left[1 - \frac{T_b}{T_{out}}\right] \qquad 6.23,$$

where, P_{in} is the initial gas pressure (85 kPa); T_{in} is the initial temperature at which the which the heat-pump accepts the waste heat exhausted from the effluent gas disengager ($T_{in} < T_b$ because of the latent loss from the gas disengager); T_{out} ($T_{out} > T_b$) is the

final temperature of the working fluid emerging from heat-pump. Rate of heat recovery (Q_{hp}) from the heat-pump is defined as follows:

$$Q_{hp} = W_{hp} + Q_{waste} \qquad 6.24.$$

The heated gas flux generated by the heat-pump can be estimated as follows (http://charming.awardspace.com/heat_pump/heat_pump_html) :

$$Q_{hp} = \frac{P_{in}\frac{G_T}{60}}{T_{in}} \cdot \frac{1}{k-1}[T_{out} - T_{in}] \qquad 6.25,$$

where,

$$k = \frac{C_p}{C_v}$$

and where, C_p is the molar specific heat(J/K/mol) at constant pressure for the working fluid mixture, and C_v is the molar specific heat (J/K/mol) at constant volume for the working fluid mixture. The efficiency of the heat recovery through the gas compression process can be defined as follows

$$\eta_{hp} = \frac{Q_{hp}}{W_{hp}} = \frac{T_{out}}{T_{out} - T_{in}} \qquad 6.26.$$

Transformation efficiency of electrical energy into heat energy by a heat-pump is in fact extremely high, with efficiencies of η_{hp} > 447 % (van Groenestijn *et al*., 2002 and http://charming.awardspace.com/heat_pump/heat_pump_html).

Additional heat energy can also be recovered from the heated effluent overflow lost from the effluent gas disengager and recycling through a heat exchanger. The heat exchanger can be either used for heating up the nutrients stored in the nutrient supply reservoir or used in counter-current (tube-shell) configuration with regard to the influent feed line. The quantity of heat recovered from the effluent overflow can be estimated as follows:

$$Q_{so} = C_{pw} F_{ir} [T_{in} - T_a] = Q_i - \lambda E - Q_{H2O} - Q_{H_2} - Q_{CO_2} \qquad 6.27.$$

6.3. Results and Discussion

6.3.1.Effect of temperature on pH and % H_2

For all bioreactor experiments (R1 to R5) the pH in the bioreactor was not controlled by any acid or base titrations. Under continuous nutrient supply the pHs for all bioreactor experiments converged onto a stable steady-state pH for the duration of the bioreactor operation (Table 6.2). For the majority of bioreactor experiments when the temperature was between 60 ^{o}C and 70 ^{o}C the average pH was 7.09 (see R1, R2, R4, R5 in Table 6.2) and when the temperature was less than 60 ^{o}C the steady-state pH fell to an average value of 5.67 (see R3, R4, R5 in Table 6.2). It is safe to conclude that in general with the type of *AFGB* system used in this study that the steady-state pH values were strongly correlated with temperature. Similarly, the % H_2 contents for the different *AFGB* configurations all converged onto steady-state values that were also strongly correlated with temperature. In the case of % H_2, when the temperatures were between 60 ^{o}C and 70

Table 6.3: Summary of bioreactor operation and performance data for different high **performance AFGB** systems**.**

Bioreactor system	Substrate	Concentra - tion	T	HRT	pH	%H_2	*HP*	*HY*	*SHP*	Bioreactor volume	Bacterial granule biomass density	References
		(g/L)	(° C)	(h)			(mmol H_2/L/h)	(mol H_2/ mol glucose)	(mmol H_2/g/h)	(L)	(g/L)	
BF AFBR	glucose	10	37	0.25	5.5		310.7	1.71	8.96	0.6[a] 1.4[b]	61 – 65[c] 34 – 37[d]	Zhang *et al*., 2008a&b
GS AFBR	glucose	10	37	0.25	5.5		269.8	1.66	8.77	0.6[a] 1.4[b]	61 – 65[c] 34 – 37[d]	Zhang *et al*., 2008a&b
UASB	sucrose	20	60	0.75	5.0	42	152.5	1.3	9.53	0.22	16	O-Thong *et al*., 2008
CIGSB SAC	sucrose	17.8	35	0.5	6.7	38.1	299.6	1.52	11.45	1.0	26.1	Lee *et al*., 2004
CIGSB CAC	sucrose	17.8	35	0.4	6.7	35.6	288.6	1.19	11.57	1.0	26.1	Lee *et al*., 2004
CIGSB	sucrose	17.8	30	0.5	6.7	34.9	160.6	1.19	5.44	0.88	30 - 40	Lee *et al*., 2005
CIGSB	sucrose	17.8	35	0.5	6.7	40.5	280.8	1.56	7.73	0.88	30 - 40	Lee *et al*., 2005
CIGSB	sucrose	17.8	40	0.5	6.7	40.1	313.1	1.58	9.12	0.88	30 - 40	Lee *et al*., 2005
CIGSB	sucrose	17.8	45	0.5	6.7	32.9	215.8	1.33	7.11	0.88	30 -40	Lee *et al*., 2005
CIGSB	sucrose	17.8	35	0.5	6.7	40.5	280.8	1.56	7.64			Lee *et al*., 2006
CIGSB[e]	sucrose	17.8	35	0.5	6.7	41.7	380.6	1.96	9.50		40	Lee *et al*., 2006
AFGB HER	sucrose	17.8	45	0.37	5.4	45	298.7 (at 85 kPa)	1.24	20.07	5.0[b] 10.5	19.5	Ngoma *et al*., 2011
AFGB HER	sucrose	17.8	70	0.37	5.5	67	507.5 (at 85 kPa)	2.2	29.59	5.0[b] 10.5[f]	22.7	Ngoma *et al*., 2011

AFBR: anaerobic fluidized bed reactor; AFBR GAC: AFBR with bacteria biofilm attached to granulated activated carbon; AFGB HER: anaerobic fluidized granular bed reactor with high rate effluent recycling; AFBR : biofilm anaerobic fluidized bed reactor; CIGSB CAC: carrier induced granular sludge bed with cylindrical activated carbon; CIGSB SAC: with spherical activated carbon; AFBR granular sludge anaerobic fluidized bed; UASB: upflow anaerobic sludge bed. a: bioreactor working volume corresponding to bed biofilm or granular height; b: bioreactor working volume; c: biomass in corresponding to bed height; d: biomass in total bioreactor working volume; e: CIGSB with bed agitation; f: total bioreactor system volume.

^{o}C the average % H_2 was 64.4 (see R1, R2, R5, in Table 6.2). When temperatures were below 60 ^{o}C the average % H_2 fell to 43.3 (see R3, R4, R5 in Table 6.2). Reported pHs for the operation of high rate granular bed or biofilm bioreactors ranged from 5.5 to 6.7 (Table 6.3). In the case of % H_2 content, the reported values ranged from 32.9% to 45% for high rate mesophilic bioreactors (Table 6.3). Reported % H_2 contents for high rate thermophilic bioreactors ranged from 42% to 67 % (Table 6.3).

6.3.2. Interrelationships between temperature, pH, % H_2 and HY

In some cases an increase in both pHs ($\geq$ 7.0) and %H_2 contents ($\geq$ 60 %) occurred when temperatures were increased from 45^{o}C to within the range of 60 ^{o}C to 70 ^{o}C (R1

and R 3 in Table 6.2) . However, in these cases high pHs and high % H_2 contents were not associated with *HYs* becoming equal to or greater than 3.0 mol H_2/mol glucose (Tables 6.2 and 6.3). Therefore the application of thermophilic temperatures did not appear to be a sufficient condition for achieving high *HYs* values (Tables 6.2 and 6.3).

6.3.3. Interrelationships between temperature, V/F_{er}, pH, % H_2 and HY

As the V/F_{er} (min) values were reduced the *HY* increased at all temperatures (see R5 results in Table 6.2 and combined R1, R2, R3, R4, and R5 results in Figure 6.2). When

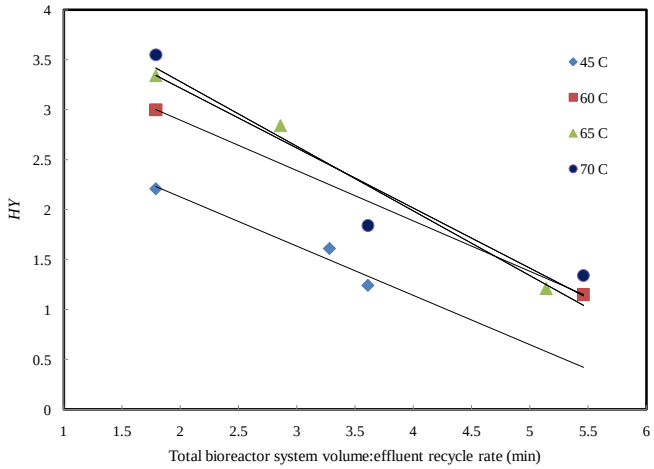


Figure 6.2: The effect of increasing temperature, total *AFGB* volume and degassed effluent recycle rate (L/min) on the hydrogen yield (*HY*) in mol H_2/mol glucose. Total *AFGB* volume: $V = B + S + G + P$, in L. Total bioreactor system volume: effluent recycle rate (min) = V/F_{er}, where F_{er} is the degassed effluent recycle rate in L/min.

V/F_{er} was reduced to 1.79 min *HY* increased to values equal to greater than 3.0 mol H_2/mol glucose, but only at thermophilic temperatures. Also at an V/F_{er} value of 1.79 min the steady-state pHs increased systematically from 5.6 to 7.8 (See R5 in Table 6.2). In addition, at an V/F_{er} value of 1.79 min the steady-state %H_2 content increased from 40% to 62 % (see R5 in Table 6.2). In this particular case, where V/F_{er} was reduced to the lowest possible value (3.2 L/min) allowable for stable bioreactor operation under the specific bioreactor dimensional configurations (R5, Table 6.1), a strong correlation between temperature, pH, % H_2 and *HY* emerged (Table 6.4) for the R5 system. There results confirm that the total volumetric size of bioreactor configuration (V) in relation to the bioreactor's mode of operation in terms of recycled effluent rates (F_{er}) are fundamental principles with regard to improving *HY*.

6.3.4. Interrelationships between temperature and HP

It has been demonstrated that the maximization of *HP* in *AFGB* systems depended on bacterial biomass density, temperature, influent rate, and degassed effluent recycle rates (Ngoma *et al*., 2011 and Lee *et al*., 2005). Volumetric hydrogen productivities in different *AFGB* experiments ranging from 152.5 to 506 mmol H_2/L/h have been readily achieved (Table 6.3). In the set of experiments undertaken in this study the highest *HPs* were achieved under thermophilic temperatures (Table 6.2).

Table 6.4: Energy balances for biohydrogen production corresponding to the different bioreactor configurations (R1, R2, R3, R4, and R5) and operational temperatures.

R	T	H_2 produ-ction	H_2 energ-y	Influent pump	Effluent recycle pump	Influent heating	Latent heat loss	H_2O vapou-r heat flux	H_2 heat flux	CO_2 heat flux	Total energy loses	Heat energy Recove-red	Net energ-y loss	Net energy output or net work done by the bioreact-or	Volumet-ric power output	Energy efficienc-y
	T_b	G_H	B_{H2}	P_{ir}	P_{er}	Q_i	λE	Q_{H2O}	Q_{H2}	Q_{CO2}		Q_{eo}		W_{net}	W_{net}/B	W_{net}/B_{H2}
	(°C)	(L H_2/h)	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(W)	(W/L)	%
			Eq (10)	Eq (14)	Eq (12)	Eq (11)	Eq (17)	Eq (20)	Eq (21)	Eq (22)		Eq (27)		Eq (9)		
R1	60	39.35	139	-1.14	-30.95	-549.94	-4.97	-0.13	-0.38	-0.16	-587.67	544.30	-43.37	95.63	19.13	68.8
R1	70	49.93	176	-1.14	-30.95	-707.06	-8.81	-0.31	-0.62	-0.19	-749.08	697.13	-51.95	124.05	24.81	70.5
R2	65	32.26	114	-0.81	-30.95	-446.93	-6.19	-0.19	-0.36	-0.21	-485.64	439.98	-45.66	68.34	13.67	60.0
R3	45	20.74	73	-1.14	-30.95	-314.25	-1.77	-0.03	-0.11	-0.08	-348.33	312.26	-36.07	36.93	7.39	50.6
R3	70	61.28	216	-1.14	-30.95	-707.06	-10.15	-0.35	-0.76	-0.19	-750.60	695.61	-54.99	161.01	32.20	74.5
R4	45	19.75	70	-0.38	-28.30	-104.75	-1.93	-0.03	-0.11	-0.08	-135.58	102.60	-32.98	37.02	11.32	52.9
R4	65	22.92	81	-0.38	-30.95	-209.50	-4.73	-0.15	-0.25	-0.16	-246.12	204.21	-41.91	39.09	11.95	48.3
R5	45	10.79	38	-0.38	-28.30	-104.75	-1.11	-0.02	-0.06	-0.05	-134.67	103.51	-31.16	6.84	2.09	18.0
R5	60	18.14	64	-0.38	-28.30	-183.31	-2.26	-0.08	-0.18	-0.11	-214.62	180.68	-33.94	30.06	9.19	47.0
R5	65	21.35	75	-0.38	-28.30	-209.50	-3.89	-0.12	-0.24	-0.14	-242.57	205.11	-37.46	37.54	11.48	50.1
R5	70	22.05	78	-0.38	-28.30	-235.69	-4.83	-0.16	-0.27	-0.17	-269.80	230.26	-39.54	38.46	11.76	49.3

6.3.5. Interrelations between HP and HY

In the bioreactor configuration R5 high *HPs* (231.3 mmol H_2/L/h) and high *HYs* (3.55 mol H_2/mol glucose) were simultaneously achieved (Table 6.2). Efficient removal of H_2 from the bioreactor was physically achieved by means of recycling of degassed effluent at a high flow rate through the bioreactor bed (Ngoma *et al.*, 2011). High rates of degassed effluent recycling appeared to have removed a major thermodynamic constraint preventing the simultaneous achievement of high *HPs* and high *HYs* in a bioreactor with a high microbial biomass density.

6.3.6. Energy balance analysis

The simultaneous maximization of *HPs* and *HYs* has become a practical possibility with bioreactor configuration R5 (Table 6.2), however, the ultimate viability of the R5 based process depends on the achievement of an overall net positive energy gain with regard to the bioreactor's overall input-output energy balance. A positive net energy balance would be an essential process requirement in the application of dark fermentative H_2 production for electricity generation (Das, 2009; Hallenbeck, 2009; Perera *et al*., 2010; Perera *et al*., 2011). Application of an energy balance model to the 5 different bioreactor systems confirmed the possibility (van Groenestijn *et al*., 2002) that with insulation and efficient waste heat recycling thermophilic dark biohydrogen generation can deliver positive net work for electricity generation (Table 6.4). Using the thermophilic energy balance model developed in this study, the calculated net positive volumetric power output (W_{net}/B) ranged from 2.04 to 32.2 W/L (Table 6.4). Energy conversion efficiencies (W_{net}/B_{H_2}) ranged from 18% to 74.5% (Table 6.4). With the additional recovery of heat energy losses from the effluent gas disengage resulting from the application of a heat-pump (van Groenestijn *et al*., 2002), energy conversion efficiencies presented in Table 5.4 could be further increased, possibly to values greater that 75%, thereby increasing the overall energetic viability of dark thermophilic biohydrogen production in *AFGB* systems.

Application of external work in the form of heat is a necessary but not a sufficient condition for increasing *HY* in *AFGB* systems. In order to remove H_2 from the bulk fluid phase in which the granules are suspended and thereby increase the thermodynamic

gradient driving the H_2 generating reaction the recycling of degassed effluent above a critical flow velocity has proved to be an essential or obligatory condition for the simultaneous achievement of high *HYs* and high *HPs*. However, shifting the equilibrium of the H_2 generation reactions further to the right in order to achieve the high *HYs* and high *HPs* has involved the performance of additional hydrodynamic work on the bioreactor system equal to P_{er} (see equation 6.12). The calculated rate of pumping work necessary for the simultaneous achievement of high *HPs* and high *HYs* in the R5 bioreactor system was 28.3 W. This would be equivalent to the application of a volumetric power input (P_{er}/B) of 8.65 W/L, which could also be taken as an index of the hydrodynamic power density necessary to remove H_2 from the bulk liquid phase in which the granules are suspended, with regard to an *AFGB* system.

6.4. Conclusions

Recently, significant advances have been in the development of thermophilic bioprocesses based on either immobilized bacterial cultures or bacterial granules for biohydrogen production (Akutsu *et al*., 2009; Keskin *et al*., 2011; Konjan *et al*., 2011; Nissilä *et al*., 2011). The results of this study has helped to identify a number of fundamental bioreactor system design and operation principles which could be adapted to any thermophilic bioprocesses using immobilized bacteria cultures, thereby increasing the *HP*, *HY* and W_{net} of these biohydrogen generation systems. It is hoped that the continued increases in the space/time yields (*STYs*) per unit bioreactor volume will

further encourage the commercialization of thermophilic based biohydrogen production systems.

CHAPTER SEVEN

7.1. CONCLUSION

In this study the granules used in the *AFGB* system were comprised of an undefined anaerobic thermophilic multispecies consortium of bacteria, and the *AFGB* system was operated as a chemostat under increasing dilution rates (substrate supply rates) and increasing degassed effluent recycle rates (degassing rates). This stimulated the growth, development and enrichment of thermophilic granules formed from a multispecies bacterial consortium. It was interesting to note that a thermophilic bacterial consortium could be derived from mesophilic inoculum. The influence of bacterial densities on *HP*, *HY* and *SHP* was investigated. It was observed that *HP* increased with bacterial biomass density. However, both *HY* and *SHP* decreased with bacterial biomass density as degassed influent recycle rates were reduced. In addition, the observation of constant hydrogen content at all *HRTs* hydrogen retention times further suggests a fairly stable community structure of the H_2-producing bacterial consortium, which was mainly maintained and retained in the granular sludge. Stability of bioreactor operation at high biomass densities was successfully achieved by means of sedimentation column or solid-liquid separator column connected to the top of the bioreactor. However, this did have draw backs as it increased the total volume of the bioreactor system which in turn resulted in a decline in the H_2 production efficiency. This situation could have been remedied by increasing the degassed effluent rate to values greater than what was possible at the time, that is greater than 3.5 L/min. The alternative experimental solution was to reduce both granular bed height and bioreactor system volume.

This study carried the latter modification. A reduction in system volume and with corresponding relative increase in the degassed effluent recycle rate had dramatic consequence for increasing both productivity and efficiency of hydrogen production. Therefore the modification of the bioreactor design and process operation for the *AFGB* system that was initially developed as described in Chapter 3 resulted in a significant improvement in H_2 output. In the improved design and operations the merits of rapid start-up, high organic loading tolerance, excellent biomass retention, were still maintained. In addition, there was no trade-off between high productivity and efficiency. At a high rate of H_2 productivity, such as 231.3 mmolH_2/L/h, high H_2 yields of 3.55 molH_2/mol glucose became possible with these improvements. Proof of concept for the *AFGB* system was achieved and the prospects for the commercial viability of H_2 as clean energy fuel have become more favourable as consequence of this study.

A fundamental condition for the viability of *AFGB* systems is the efficiency of H_2 removal from the interstitial spaces between the granules within the bioreactor bed. Efficient removal of H_2 from the bioreactor was physically achieved by means of recycling of degassed effluent at a high flow rate through the bioreactor bed (Ngoma *et al*., 2011: Obazu *et al*., 2012). High rates of degassed effluent recycling appeared to have removed a major thermodynamic constraint preventing the simultaneous achievement of high *HPs* and high *HYs* in a bioreactor with a high microbial biomass density.

In addition, the reduction of hydraulic retention times for degassed effluent recycling through the fluidized granular bed resulted in very high volumetric hydrogen productivities at themophilic temperature. Usually when the degassed effluent recycle

rate is reduced the likelihood for gas sludge piston induced granular bed washout increases. However, due to the solid-liquid separator (Figure 3.1) no granular bed wash occurred at the lower effluent recycle rates. However, every under performance with regard to hydrogen generation efficiencies for granular bed bioreactors were remedied by simply increasing the rate of degassed effluent recycling.

While thermophilic temperatures proved to be a necessary condition, it was not sufficient condition for achieving simultaneously both high *HPs* and high *HYs*. However, in addition to thermophilic temperature other kinds of bioreactor design and operational interventions were found to be necessary, for example, reductions in hydraulic retention time and increases in degassed effluent recycling rate. Clearly hydraulic retention time for degassed effluent recycling was a critical factor for the simultaneous achievement of high *HPs* and high *HY*. It was discovered that a reduction in the total volume of bioreactor system relative to increasing rates of degassed effluent recycle was a necessary condition for the simultaneous achievement of both high *HPs* and high *HYs*. Thus at thermophilic temperatures any increase in the bioreactor system volume should also be accompanied by a concomitant increase in the rate of degassed effluent recycling so the *HRT* always remained below the critical edge necessary for the simultaneous achievement of high *HPs* and high *HYs*.

In summary, the successful outputs of this study include the following:

1. Significantly improved design configurations and operational conditions of bioreactor systems which together facilitate enhanced biohydrogen production efficiencies from sucrose.
2. Induction, development and growth of thermophilic bacterial granules consisting of a mixed consortium
3. Achievement of high volumetric bacterial biomass densities.
4. Operation of the bioreactor low *HRTs* and high organic substrate loading rates.
5. Operations of the bioreactor at high effluent recycle rates.
6. Proof that the bioreactor was energy efficient through a theoretical energy balance study of the bioreactor system.
7. The achievement of the best possible procedure that would maximize optimal mass production of hydrogen at a reduced and affordable cost.
8. The durability of the anaerobic fluidized granular bed reactor at high temperature was achieved.
9. To develop a strategy for selective growth of hydrogen producing bacteria.
10. It was predicted by the hypothesis outlined in considerable detail in Chapter 3 that a simultaneous increase of both *HP* and *HY* was experimentally achieved by increasing temperature of the bioreactor and by increasing the de-gassed effluent recycle rates through the bioreactor bed.
11. This entire study showed that suspended solid retention time (for suspended granules) was completely decoupled from hydraulic retention times, which

means that all suspended solids were retained indefinitely in the bioreactor vessel as a fluidized granular bed.

In conclusion, these experiments have reproduced and confirmed all the conditions that are necessary for achieving a high rate thermophilic dark fermentation bioprocess for H_2 generation. Hence, the study confirms the hypothesis that both high *HPs* and *HYs* can be simultaneously achieved. It also provides compelling evidence that biohydrogen production via the *AFGB* system has the greatest chance of success in the biohydrogen economy.

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