Biohydrogen Production by Facultative and Obligate Anaerobic Bacterial Consortia in Fluidized Bioreactor

A thesis submitted
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DOCTOR OF PHILOSOPHY

BY

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DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of doctor of philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at any other university.

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Lubanza Ngoma Date

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DEDICATION

This thesis is dedicated to my daughter Divine Lubuya, Emmanuel Tshimanga and my wife Susan Tshiame. They have been my inspiration and motivation throughout this work. I love you.

LIST OF OUTPUTS

During my PhD programme I have generated outstanding results which resulted provisional patent that was filed by Wits Enterprise Ltd.

The following articles from the research outlined in this thesis has been published or presented in scientific journals and/or conferences.

- **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 Technology*. **102:** 8986–8991. Published.

- **L. Ngoma**, Franklin O Obazu, V.M. Gray (2011). The influence increasing temperatures on hydrogen productivities and hydrogen yield. Elsevier Editorial System(tm) for *International Journal of Hydrogen Energy*. Submitted.

- Franklin O Obazu, **L. Ngoma**, V.M. Gray (2011). Interrelationships between bioreactor volumes, effluent recycle rate, temperature, pH, %H2, hydrogen productivity and hydrogen yield with undefined bacterial cultures. Elsevier Editorial System(tm) for *International Journal of Hydrogen Energy*. Submitted.

- Franklin O Obazu, **L. Ngoma**, V.M. Gray (2011). Stability of biohydrogen production at extreme thermophilic (70^{o}C) temperatures by an undefined bacterial culture. Elsevier Editorial System(tm) for *International Journal of Hydrogen Energy*. Submitted.

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LIST OF ABBREVIATIONS

ACIA: Arctic Climate Impact Assessment.

AFGB: Anaerobic fluidized granular bed bioreactor.

BEB: Bioreactor Energy Balance Hypothesis.

BM: bacterial biomass

BMD: Bacterial biomass densities.

CAC: Cylindrical activated carbon:

CFCs: Chlorofluorocarbons.

CH4: Methane.

CIGSB: Carrier-induced granular sludge bed reactor.

CO: Carbon monoxide.

CO_2: Carbon dioxide.

COD: Chemical oxygen demand.

CSTR: Continuous starred tank reactor.

CSTR: Granule-based continuous stirred tank reactor.

DGGE: Denaturing gradient gel electrophoresis.

DNA: Deoxyribonucleic acid.

ECP: Extracellular products.

EJ: Exajoule.

EPSs: Extracellular polymeric substances

FCM: Fuel cell - bioreactor volumetric.

GC: Gas chromatography.

H_2: Hydrogen.

H_2O: Water vapours.

H_2S: Hydrogen sulphides.

HCl: Hydrochloric acid.

HP: Hydrogen productivity.

HPLC: High-performance liquid chromatography

HRT: Hydraulic retention time

HYs: Hydrogen yields.

IASC: International Arctic Science Committee.

IPCC: Intergovernmental panel on climate change.

KJ: Kilo-joule.

L/h: Litre per hour.

Mmol: miles mole.

N_2: Nitrogen gas

N_2O: Nitrous oxide.

NCBI: National centre for biotechnology information.

OLR: Substrate loading rates.

Pa: Pascal

PCR: Polymerase chain reaction.

PHP: Potassium hydrogen phthalate.

PVC: Polyvinyl chloride

rRNA: Ribosomal ribonucleic acid.

SBRs: Sequencing batch reactors.

SCFAs: Short chain fatty acids.

SEM: Scanning electron microscopy.

SF_6: Sulfur hexafluoride

SHP: Specific hydrogen production rate

STYs: Space/time yields.

UASB: Upflow anaerobic sludge bed.

UV: Ultraviolet.

VFAs: Volatile fatty acids.

VSS: volatile suspended solids

ABSTRACT

Biological production of hydrogen gas has received increasing interest from the international community during the last decade. Most studies on biological fermentative hydrogen production from carbohydrates using mixed cultures have been conducted in conventional continuous stirred tank reactors (CSTR) under mesophilic conditions. Investigations on hydrogen production in reactor systems with attached or self-immobilized microbial growth have also appeared recently in the literature. These investigations on attached or self-immobilised bacteria involve hydrogen production in the mesophilic and thermophilic temperature range. The present study investigated the design and operational features of anaerobic fluidized granular bed bioreactor (AFGB) system which would facilitate the simultaneous achievement of high productivities (HPs) and high hydrogen yields (HYs).Where high HPs is greater than 120 mmol H_2 /(L.h) and HYs greater than 4 mol H_2/mol glucose. Theoretical maximum yield for an exponentially growing non-granulated bacterial monoculture will always be less than the thermodynamic maximum of 4 mol H_2 /mol glucose: $C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 4H_2 + 4H^+ + 2HCO_3$. The design features included reducing the total non-working or dead volume of bioreactor system. The operational improvements included application of thermophilic temperatures and high rates of de-gassed effluent recycling through the fluidized granular bed. An example of an optimal ratio of effluent recycle rate (R) to bioreactor working volume (V) was (3.0 L/min)/(3.2 L/min) = 0.94 minutes. Under conditions where temperatures were maximised and V/R were minimized the HPs increased to 21.58 L H_2 /h. Also under these conditions the HYs increased above 3.0 mol H_2/mol glucose. Specific hydrogen productivity for the fluidized granular bed increased from 0.25 L H_2 / (g BM.h) or 8.83 mmol H_2 / (g BM.h) at 45 ^oC to 0.525 L H_2 / (g BM.h) or 18.03 mmol H_2 / (g BM.h) at 70 ^oC. A 3.64 fold increase in hydrogen yield occurred with an increase in temperature from 45 ^oC to 70 ^oC.

When expressed in terms of glucose, this represents an increase from 1.34 mol H_2 /mol glucose to 4.65 mol H_2 /mol glucose. Finally, an evaluation of the net energy production by the AFGB system revealed a positive energy balance, making thermophilic biohydrogen production energetically viable from a commercial perspective.

Key words: Biohydrogen, fluidized bed, granules, hydrogen productivity, hydrogen yield, mesophilic, thermophilic.