Sustenance of Dark Fermentative Hydrogen Production by an Undefined Bacterial Culture

Franklin O. Obazu*Ayo S Afolabi, *Member, IAENG*, Michael O. Daramola, Vincent M. Gray

***Abstract*— Thermophilic bacterial granules adapted to 70°C were rapidly generated from an undefined bacterial cultures obtained from mesophilic sources. The H_2 generation process goals were assessed by considering two factors Firstly, whether a net positive net energy balance can be attained at thermophilic temperatures, and high effluent recycle rates. Secondly, whether the volumetric hydrogen productivities were sufficient to drive a 5 kW fuel cell when scale-up to 1 m^3.**

***Keywords* —** *mesophilic, thermophilic, temperature, biohydrogen, recycle rates, bioreactor system.*

I. Introduction

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. For an anaerobic bacteria granular fluidized bed bioreactor system (AFBR) with degassed effluent recycling [1], the energy balance model for computing the net work done under thermophilic temperatures with respect to H_2 energy generation can be estimated from the following energy balance relationship [2]:

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

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

where, W_{net} is the network 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} is the power required for recycling the degassed effluent through the fluidized granular bed; Q_i , 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 , the radiant energy emission flux from the bioreactor and gas-disengager surfaces; H , the free and forced convective or sensible heat flux from the bioreactor and gas-disengager surfaces; λE , 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, respectively. W_{hp} is the electrical power required for the operation of the heat-pump; Q_{hp}, the heat energy delivered from the heat-pump; Q_{eo}, the heat energy recovered from the effluent overflow lost from the effluent gas disengager; and h is the time (in hour).

If W_{net} is negative for H_2 generation for an *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 thermophilic H_2 generating system [2]. 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 system 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 [2]. 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 used either 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 [2]:

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

In the bioreactor energy balance equation (Eqn. 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 (Eq. 3) and

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Franklin O. Obazu* Michael O. Daramola, are both with the School of Chemical & Metallurgical Engineering ,Faculty of Engineering and the Built Environment, University of the Witwatersrand, Wits 2050, Johannesburg, South Africa. (*Corresponding author: phone: +27 73 974 0668; e-mail: obazuf@gmail.com)

Ayo S Afolabi is with the the Department of Civil and Chemical Engineering, University of South Africa, Florida Campus, Johannesburg, South Africa

Vincent M. Gray is with the School of Molecular and Cell Biology, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa.

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insulation of the bioreactor system will increase the overall energy balance efficiency of the 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 a H_2 supply rate of 2900 L H_2 /h [3]. 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 [1-2,4,5,6]. Against this background, in this study we evaluate whether the aforementioned process goals or target can be reached in an AFGB system.

II. Methodology

A. Medium

An Endo formulation [7,8] was used as the nutrient medium for inoculum preparation and for the bioreactor experiments. The medium contained 17.8 g sucrose/L together with 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.

B. 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.

C. Bioreactor design and set-up

The bioreactor system consisted of the following four (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 [2,6]. 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 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.

D. 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 [4]. 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. Then the HRT was 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

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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°C.

E. 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 [1-2,6]. 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 [1,6].

F.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 40 µl. The following formula (Eq (4)) was used for converting total bioreactor gas flux (L/h) to mmol H_2/h:

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

where, $\Delta H_2/\Delta t$ is the mmol H_2 /h; P , the atmospheric pressure (kPa); ($\%H_2^{GC}$), the percentage hydrogen content from GC measurements; $\Delta V/\Delta t$, the volumetric flow rate (L/h) of total gas production from the gas meter measurements. R is the gas constant (8.314 J/(K.mol)) and T, 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 [9].

III. Results and Discussion

A. 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 49% at 55°C to 71% at 70°C (Fig. 1). Similarly, total H_2 also increased with increasing temperatures, from 21.5 L/h at 55°C to 36.6 L/h at 70°C.

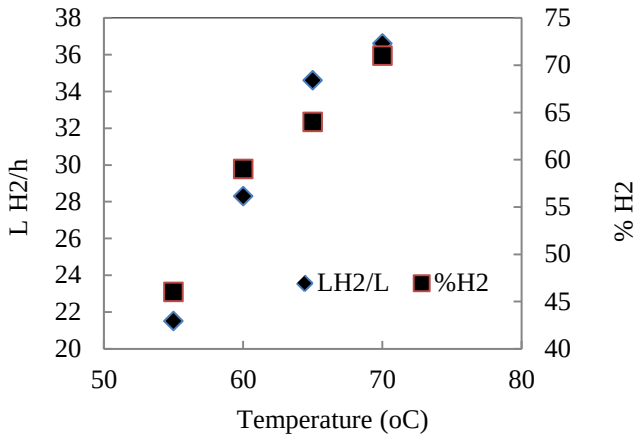

Fig. 1: Percentage hydrogen rates and total H_2 production rates with temperature increase. Total hydrogen production was a factor effluent recycle rates and temperatures However, pressure of 85 kPa were calculated at ambient temperature.

Hydrogen yield followed the same trend as % H_2 and total H_2 production, by increasing in response to increasing temperature (Fig. 2).

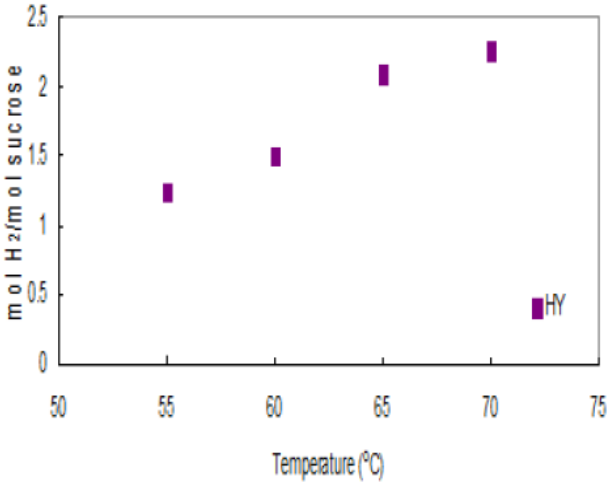

Fig. 2: Hydrogen yield increase in response to temperature increase correspondingly and degassed effluent recycle rate was 3.5 L/min.

B. Net energy output.

Table 1 shows the net hydrogen energy output at different temperatures. Net hydrogen energy hydrogen energy output was defined as follows:

$$E_{output} = B_{H2} + Q_{rec} - E_{input} \qquad \text{(v)}$$

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% to78%. The capacity for the bioreactor to generate positive work will be dependent on the efficiency heat energy recovery.

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TABLE I
NET HYDROGEN ENERGY OUTPUT AT 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_0	F_{in}	F_{er}	G_H	B_{H2}	P_{in}	P_{er}	Q_i	λE	Q_{H2O}	Q_{H2}	Q_{CO2}	E_{input}	Q_{rec}	E_{output}
°C	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

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

C. 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°C), 5660 L H_2/m^3 (60°C), 6920 L H_2/m^3 (65°C), 7320 L H_2/m^3 (70°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 [3].

IV. Conclusions

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.

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