Biohydrogen Production by Bacterial Granules Adapted to grow at Different Thermophilic Temperatures

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

***Abstract*—Bacterial granules grown from mesophilic inoculant were adapted to generate H_2 from sucrose under a range of thermophilic temperatures (50, 55, 60, 65, 70°C). In theory under thermophilic temperatures the "Thauer limit" of 4.0 mol H_2/mol glucose should be achievable. However, while thermophilic temperatures may be a necessary condition for high yields, this study shows that it was not a sufficient condition. The stability and maintenance of dark anaerobic biohydrogen production in the fluidized granular bed bioreactor was achieved.**

Keywords *— biohydrogen production, fluidized bed, bacterial granules, thermophilic. mesophilic*

I. Introduction

The majority of recent reports on hydrogen production by bacterial granules have involved mesophilic systems [1-3]. Volumetric hydrogen productivities (HPs) ranging from 7.3 L H_2/(L.h) to 9.3 L H_2/(L.h) have been achieved for mesophilic fluidized bacterial granular bed bioreactors [1,4]. However, the hydrogen yields (HYs) for the above mesophilic fluidized granular bed bioreactors did not exceed 2.0 mol H_2/mol glucose when the HPs reached their maximal levels [1,4,5,6]. In theory under thermophilic temperatures the "Thauer limit" of 4.0 mol H_2/mol glucose should be achievable [7,8]. Hydrogen yields of 4.0 mol H_2/mol glucose have been achieved with a monoculture of *Caldicellulosiruptor owensis* OLT under thermophilic conditions and with N_2 gas sparging [9]. It is not clear whether a mixed anaerobic thermophilic bacterial culture in a continuous anaerobic fluidized granular bed bioreactor (*AFGB*) system would achieve hydrogen production efficiencies exceeding 2.0 mol H_2/mol glucose. Mixed thermophilic anaerobic bacterial consortia have been obtained by adapting mesophilic sewage sludge to higher temperatures [10,11]. In this paper we have confirmed that an undefined mixed bacterial culture obtained from mesophilic sources could be adapted to form granules at thermophilic temperatures. The objective of the study was induce granule formation from a bacterial cultured adapted to grow at a thermophilic temperature of 70°C, and then to investigate whether the various biohydrogen production parameters such as the HPs, HYs, % H_2 and specific hydrogen productivity (SHP) obtained for a thermophilic AFGB system would be similar or different to those reported for mesophilic AFGB systems.

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II. Methodology

A. Inoculum preparation and Medium

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 90ºC 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. An Endo formulation 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 [12,13].

B. 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 [14,15]. 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

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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, 60ºC and 70ºC, 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.

C. 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 [16]. 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 1 [17].

$$\frac{H_2^L}{H_2^{L*}} = \frac{HP_L}{K_H^T R T k_L} + 1 \qquad \text{(i)}$$

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 2) as follows

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

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.

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. 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

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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 [14,15,18]. 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 [15,18].

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. Equation 3 was used for converting total bioreactor gas flux (L/h) to mmol H_2/h ,

$$\frac{\Delta H_2}{\Delta t} = \frac{P\left[\left(\%H^{GC}\right)\frac{\Delta V}{\Delta t}\right]}{RT} \quad (3).$$

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 [19].

G. 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. 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°C. The temperature of the bioreactor was then dropped to 60°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.

III. Results and Discussion

A. Effect of temperature on %H_2

The entire H_2 generation process had a marked increase in % H_2 contents which was perhaps seen to be due to response to increasing temperature respectively (Fig 1).

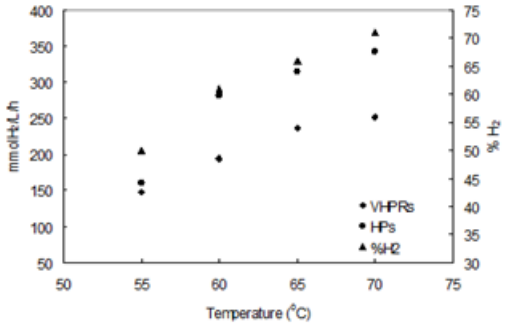

Fig. 1: Percentage H_2 with increasing temperature. As influent rates were increased from 1.3 L/min to 3.5 L/min % H_2 content increased from 50% to 71 %. This increase went across the volumetric H_2 production rates (VHPRs) and total H_2 production rates (HPs) respectively.

The influence of increasing temperature in anaerobic dark fermentation cannot be over emphasized in the sense that, from literature it has been reported that anaerobic hydrogen consumption via an H_2 uptake hydrogenase, was reduced with increasing temperature, resulting in increase in hydrogen generation [20]. Therefore, temperature is a positive factor to enhancing hydrogen generation and productivity.

B. Effect of temperature on HPs and VHPRs

With sucrose as substrate, the *AFGB* system developed in the present study established respective maxima of various volumetric H_2 production rates (VHPRs) at different themophilic temperature to be 147.64 mmol/L.h at 55°C, 194.22 mmol/L.h at 60°C, 237.02 mmol/L.h at 65°C and at 70°C it was 251.25 mmol/L.h (Fig. 1). Temperature and effluent recycle rate increase is directly proportional to high HPs and VHPRs. The total H_2 output rates for the various temperature (55°C, 60°C, 65°C and 70°C) bioreactors shows the effects of temperature and effluent recycle as it influence H_2 volumetric production rate (Fig. 1) respectively.

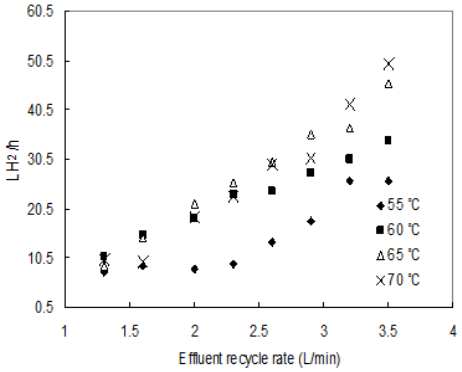

Fig. 2: Total hydrogen production under extreme thermophilic and thermophilic conditions with respect to increase in effluent recycles rates. A. Total hydrogen production in terms of LH_2/L/h (calculated at ambient pressure of 85 kPa) at various themophilic temperatures. The degassed effluent recycle rate was 3.5 L/min.

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C. Effect of HRT on VHPRs

After the initiation of granule, there was further reduction in the HRT to between 2 and 1.6 h which led to an increase in volumetric hydrogen production rates at themophilic temperature and further resulted in the rapid growth and expansion of the granular bed size. Table 1 summarizes the yields and the volumetric rates of hydrogen production (VHPRs) for continuous operations with various HRTs. No methane was detected during the course of all experiments. This suggests that the acidic pretreatment and the operation conditions used were quite effective in terminating (or inhibiting) the activity of methane forming population in the mixed bacterial consortium.

TABLE I
YIELDS AND RATES OF BIO-HYDROGEN PRODUCTION FROM PURE CARBOHYDRATES BY CONTINOUS DARK FERMENTATIONS

Organism	Carbon	HP	HY	Reactor	HRT (h)	Reference
Mixed culture	Glucose (13.7g/L)	376 mmol/L/ d	1.2 mol/mol glucose	Trickling biofilter	4–12	21
Clostridia sp.	Glucose (20 g COD/L)	59 mmol/L/ d	1.7 mol/mol glucose	CSTR	6	22
Mixed culture	Sucrose (20 g COD/L)	105 mol/h	3.47 mol/mol sucrose	CSTR	8	23
Mixed culture	Sucrose	5.10 L/h L	2.1 mol/mol sucrose	CIGSBR[b]	0.5	4
Mixed culture	Sucrose (20 g COD/L)	270 mmol/L/d	1.5 mol/mol sucrose	UASB	8	24
Mixed culture	Sucrose (20 g COD/L)	470 mmol/L/d	2.6 mol/mol glucose	SBR	4–12	25
Klebsiella oxytoca HP1	Sucrose (50 mM)	350 mL/L/h	3.6 mol/mol sucrose	CSTR	5	26
Mixed culture	Sucrose (20g COD/L)	20.8 L/L/ d	1.48 mol/mol sucrose	CSTR	2	27
C. butyricum + *E. aerogenes*	Starch (2%)	800 mL/L/ h	2.5 mol/mol glucose	CSTR	2	28
Mixed culture	Wheat starch (10g/L)	131 mL/L/ h	0.83 mol/mol starch d	CSTR	12	29
Mixed culture	Starch (6 kg starch/m3)	1497 L/m3 d	1.29 L/g starch COD	CSTR	20	30
C. termolacticum	Lactose (29 mmol/L)	2.58 mmol/L/ h	3 mol/ mol lactose	CSTR	5–35	31
Mixed culture	Sucrose (17.6g/L)	251 mmol/L/.h	2.3 mol/mol sucrose	AFGB	1.6	Current Study

b CIGBR, carrier induced granular bed reactor. VHPR, volumetric hydrogen production rate.

3.4 The effect of increasing temperature on HYs

An increase in the % H_2 content indicates an increase in the H_2 production efficiency or hydrogen yield (HY). However, the yield may be lower than the theoretical value because of utilization of substrate for cell synthesis.

D. Influence of degassed effluent recycle rate (L/min) on the hydrogen yield (HY) in molH_2/mol sucrose.

An increase in the degassed effluent recycle rate from it minimum of 1.3 L/min to 3.5 L/min resulted in high hydrogen yield (HY) within 40 hrs of experimentation (Fig. 3). And the system was stable for 50 days at varied themophilic temperature (55°C, 60°C, 65°C and 70°C). In accordance with the work Ngoma et al [18], the 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. 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.

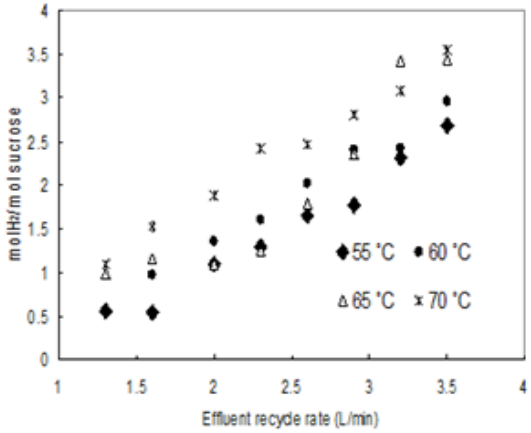

Fig. 3: As the degassed effluent recycle rate were increased from a minimum of 1.3 L/min to 3.5 L/min, consequently, resulted in high hydrogen yield (HY).

IV. Conclusions

This study shows that temperature played a paramount role among the parameters varied in controlling the H_2 production performance in the *AFGB* reactor. From the various temperature investigated above there is a common trend that H_2 production increases with a decrease in HRT and partial pressure. Finally, these results substantiate that the total volumetric size of the bioreactor configuration in relation to the bioreactor's mode of operation in terms of recycled effluent rates are fundamental principles with regard to improving HY and consequently being sufficient to drive a 5 kW fuel cell.

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