

# STABILITY OF BIOHYDROGEN PRODUCTION AT EXTREME THERMOPHILIC (70<sup>0</sup>C) TEMPERATURE BY AN UNDEFINED BACTERIAL CULTURE

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**Abstract**— 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<sub>2</sub>/L/h), %H<sub>2</sub> content and pH maintenance was readily maintained for 50 days. While volumetric hydrogen productivity increased with bacterial biomass density, both hydrogen yield (mol H<sub>2</sub>/mol glucose) and specific hydrogen productivity (L H<sub>2</sub>/g/h) declined with increasing biomass density.

**Keywords**— Biohydrogen production, Expanded bed, Bacterial granules, Biomass density, Effluent recycling, Thermophilic.

## 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<sub>2</sub> 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<sub>2</sub> supply rate of 2900 L H<sub>2</sub> /h [1]. From an STY perspective this would be equivalent to a volumetric hydrogen production rate of 2.9 L H<sub>2</sub>/(L.h) or 120 mmol H<sub>2</sub> / (L.h), irrespective of hydrogen yield (mol H<sub>2</sub>/mol glucose). Volumetric hydrogen productivities (HPs) ranging from 7.3 L H<sub>2</sub>/(L.h) to 14.8 L H<sub>2</sub>/(L.h) with hydrogen yields (HYs) not exceeding 2.2 mol H<sub>2</sub>/mol glucose have been achieved for mesophilic and thermophilic anaerobic fluidized bacterial granular bed bioreactors [2-5]. 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<sub>2</sub>/mol glucose, and maintenance of volumetric bacterial biomass densities often result in 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 [6]. 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<sub>2</sub>/(L.h) [3-10].

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 1), the accumulation of large gas bubbles or gas slugs within the expanded or fluidized bed causes a sludge piston floatation process [3], 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 impeller inserted into the fluidized granular bed [3] or by fitting a sedimentation column above the expanded or fluidized granular bed [6,11]. 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 °C) fluidized granular bed bioreactor system was investigated.

## 2. Materials and Methods

### 2.1 Medium

An Endo formulation [12,13] 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<sup>-1</sup>) : NH<sub>4</sub>HCO<sub>3</sub> 6.72, CaCl<sub>2</sub> 0.2, K<sub>2</sub>HPO<sub>4</sub> 0.699, NaHCO<sub>3</sub> 3.36, MgCl<sub>2</sub>.6H<sub>2</sub>O 0.015, FeSO<sub>4</sub>.7H<sub>2</sub>O 0.0225, CuSO<sub>4</sub>.5H<sub>2</sub>O 0.005, and CoCl<sub>2</sub>.H<sub>2</sub>O 1.24 x 10<sup>-4</sup>g.

### 2.2. Inoculum preparation

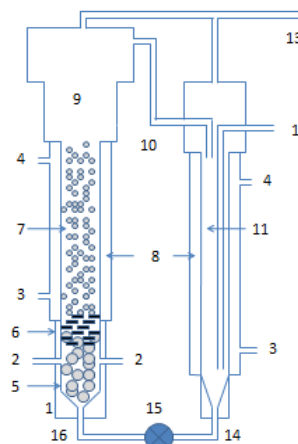
An undefined extreme thermophilic anaerobic bacterial consortium was derived from a mixture of sewage sludge and fresh cow dung. Sewage sludge was obtained from the overflow outlet of a mesophilic anaerobic digester at the Olifantsvlei wastewater treatment works (Johannesburg). Fresh cow dung was obtained 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 °C 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.

### 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 (Fig. 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. 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 diffuser 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° 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 degassed 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°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 °C and 70 °C, 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.



**Fig. 1.** Bioreactor 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 bed (5 mm) in effluent/influent diffusion and cavitation generation; 6 – activated carbon for inducing granulation; 7 – fluidized bacterial granular bed; 8 – water jacket for heater exchanger; 9 – solid-liquid separator column (sedimentation column) and effluent decanter; 10 – effluent connecting pipe to gas disengager; 11 – gas disengager tube; 12 – effluent outlet overflow pipe; 13 – gas flow pipe; 14 – effluent recycle outlet pipe; 15 – effluent recycle pump; 16 – effluent recycle inlet.

### 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°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°C.

### 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 [4,5]. 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<sub>2</sub> from the effluent through bubble production. Efficient removal of undissolved or non-solubilized H<sub>2</sub> trapped in the effluent phase by gas disengagement was expected to increase the overall biohydrogen production efficiency of the bioreactor system [4].

### 2.6. Analytical techniques

Gas chromatography was used to analyze % gas composition (H<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub>). 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<sup>-1</sup>. Sample gas injection volume was 40µl. Equation 1 was used for converting total bioreactor gas flux (L/h) to mmol H<sub>2</sub>/h ,

$$\frac{\Delta H_2}{\Delta t} = \frac{P \left[ (\% H^{GC}) \frac{\Delta V}{\Delta t} \right]}{RT} \quad (1).$$

Where,  $\Delta H_2/\Delta t$  = mmol H<sub>2</sub> /h; P = atmospheric pressure (85 kPa); (%H<sub>2</sub><sup>GC</sup>) = 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 [14].

### 2.7. Experimental design

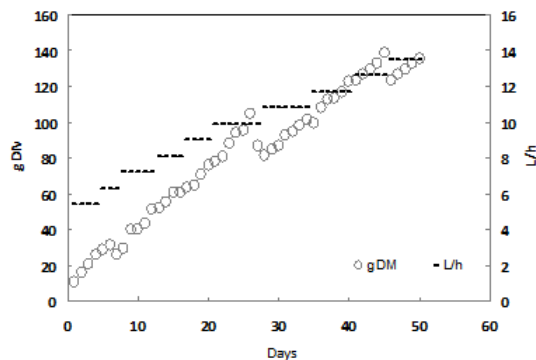
After granule formation was initiated the influent rate for the duration of the experiment was maintained at 5.4 L/h at night (14 h). During the day (10 h) the influent rates were maintained at the following rates for between 3 and 7 days: 5.4 L/h, 6.3 L/h, 7.2 L/h, 8.1 L/h, 9.0 L/h, 9.9 L/h, 10.8 L/h, 11.7 L/h, 12.6 L/h and 13.5 L/h. The bioreactor was operated in such a fashion for 50 days. 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.

## 3. Results and discussion

### 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 (Fig. 2). 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.

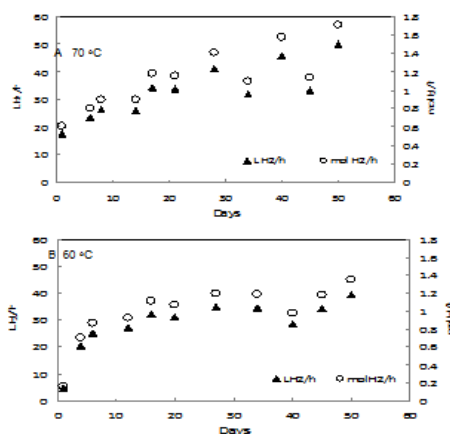


**Fig. 2.** Bacterial granular growth in response to influent supply rate.

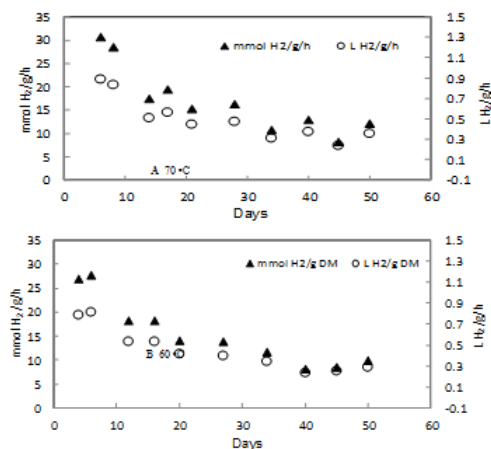
### 3.2. Influence of temperature and biomass density on biohydrogen productivity

At 70 °C total biohydrogen production increased with granule biomass, producing up to 49.9 L H<sub>2</sub>/h (Fig. 3A ), which corresponded to a volumetric hydrogen productivity of 9.98 L H<sub>2</sub>/L/h or 342.4 mmol H<sub>2</sub>/L/h, with a sucrose consumption of 91 %. These values are similar to the ones (Table 1) achieved for a fluidized granular bed system that was agitated with an impeller [3]. Specific hydrogen productivity (SHP) dropped from 30.68 mmol H<sub>2</sub>/g/h (0.89 L H<sub>2</sub>/g/h) to 12.08 mmol H<sub>2</sub>/g/h (0.35 L H<sub>2</sub>/g/h) as biomass density increased (Fig. 4A). Reported SHPs also tend to be variable (Table 1), ranging from 0.113 to 0.283 L H<sub>2</sub>/g/h for the CIGSB systems [3] or from 4.18 to 9.53 mmol H<sub>2</sub>/g/h for biofilm and granular sludge AFBR systems [15,11]. 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<sub>2</sub> /L/h to 69.6 mmol H<sub>2</sub> /L/h (Fig.

5A). Similarly, SHP dropped from 12.08 to 2.81 mmol H<sub>2</sub>/g/h when the degassed effluent recycle rate was reduced to from 3.5 L/min to 1.3 L/min. (Fig. 5B ). Reported mesophilic HPs range from 1.21 to 9.31 L H<sub>2</sub>/L/h (Table 1). Thermophilic HPs range from 3.72 L H<sub>2</sub>/L/h, calculated from the 152 mmol H<sub>2</sub>/L/h value reported by O-Thong, et al., 2008, to 14.8 L H<sub>2</sub>/L/h (506 mmol H<sub>2</sub>/L/h ) reported by Ngoma, et al., 2011 (Table 1).



**Fig. 3.** Total hydrogen production under extreme thermophilic and thermophilic conditions with respect to granular bed growth over 55 days ( see Fig. 2) . **A.** Total hydrogen production in terms of L H<sub>2</sub>/L/h and mol H<sub>2</sub>/L/h (calculated at ambient pressure of 85 kPa) at 70°C. **B.** Total hydrogen production in terms of L H<sub>2</sub>/L/h and mol H<sub>2</sub>/L/h (calculated at ambient pressure of 85 kPa) at 60°C .



**Fig. 4.** Changes in the specific hydrogen productivity with respect to granular bed growth over time (see Fig.2) at extreme thermophilic and thermophilic temperatures. Measurements given in terms of L H<sub>2</sub>/g/h and mol H<sub>2</sub>/g/h, where g is the bacterial granular biomass. **A.** Specific hydrogen productivity at extreme thermophilic temperatures. **B.** Specific hydrogen productivity at thermophilic temperatures.

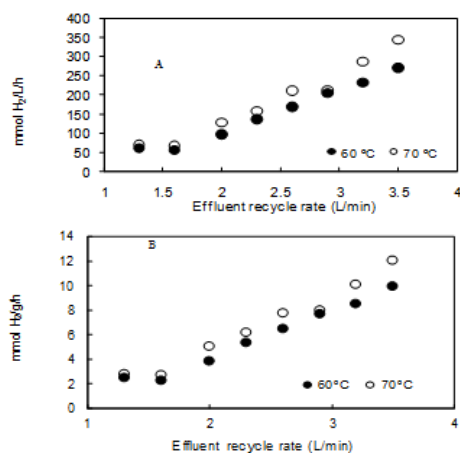
Table 1. Summary of bioreactor parameters and hydrogen productivity variables for different high performance bioreactor systems

Bioreactor System	Substrate	Concentration	T	HRT	pH	HP	HP	HP	HP	SHP	SHP	RV	BD	BF
		g/L	°C	h		L H <sub>2</sub> /L/h	mmol H <sub>2</sub> /L/h	mol H <sub>2</sub> /mol glucose	L H <sub>2</sub> /g/h	mmol H <sub>2</sub> /g/h	L	g/L		
CSTR FLOC	glucose	10	30	1	5.5	61	2.17							17
		2.5	30	10	5.5	67	0.081					2.0		
		2.5	30	2.5	5.5	72	0.27					2.0		
AFBR GAC		10	37	0.5	4.0	57.2	2.22		1.16		4.18	0.8	21.3	15
		10	37	1.0		59.2			1.10				18.7	
		20	37	1.0		59.2							21.6	
		30	37	1.0		59.2	2.36				4.34			
BF AFBR	glucose	10	37	0.25	5.5		7.6		1.71		8.96	0.8 <sup>a</sup>	61–65 <sup>b</sup>	11.6
BF AFBR	glucose	10	37	0.25	5.5		4.8		1.66		8.37	0.8 <sup>a</sup>	61–65 <sup>b</sup>	11.6
UASB	sucrose	20	60	0.75	5.0	42	3.72	0.23	1.3		9.52	0.22	16	8
AFBR	glucose	4	30	1.0	5.5	51	1.21		2.52			4.39	10	
AFBR	glucose	2	30	2.0	3.8	37	1.38		2.29			4.39	9	
CIGSB SAC	sucrose	17.8	25	0.5	6.7	38.1	7.81		1.52	0.200		1.0	26.1	2
CIGSB SAC	sucrose	17.8	25	0.4	6.7	35.6	7.06		1.19	0.202		1.0	26.1	
CIGSB	sucrose	17.8	30	0.5	6.7	34.9	3.91		1.19	0.113		0.88	30–40	16
CIGSB	sucrose	17.8	25	0.5	6.7	46.5	4.87		1.56	0.189		0.88	30–40	
CIGSB	sucrose	17.8	40	0.5	6.7	43.1	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	46.5	4.87		1.56	0.187				3
CIGSB	sucrose	17.8	25	0.5	6.7	41.7	8.31	300 (μm 501.5 kPa)	1.96	0.234	95		40	8
AFGB	sucrose	17.05	37	4.5	6.2	42.3		300 (μm 501.5 kPa)	1.17			2.88	7	
AFBR HER	sucrose	17.8	45	0.37	5.4	45	8.71	296 (μm 85 kPa)	1.34	0.491		5.0 <sup>c</sup>	18.3	4
AFBR HER	sucrose	17.8	70	0.37	5.5	67	14.8	306 (μm 85 kPa)	2.2	0.724		5.0 <sup>c</sup>	22.7	4
AFGB HER	sucrose	17.8	60	0.37	7.2	80	7.86	270 (μm 85 kPa)	0.92	0.29	9.95	5.0 <sup>c</sup>	18.35	Current study
AFGB HER	sucrose	17.8	70	0.37	7.2	68	9.98	342 (μm 85 kPa)	1.34	0.35	12.06	5.0 <sup>c</sup>	18.35	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.

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 (Fig. 1) no granular bed wash occurred at the lower effluent recycle rates.

A 10 °C drop in the temperature from the extreme thermophilic temperature of 70 °C to a thermophilic temperature of 60 °C resulted in a substantial 21.4 % decline in the total biohydrogen production, that is, from approximately 50 L H<sub>2</sub>/h to 39.3 L H<sub>2</sub>/h (Fig. 3 B ). Similarly, a drop in the temperature to 60 °C also resulted in substantial declines in the HP and SHP relative to the 70 °C treatment (Figs. 4 and 5), also indicating that the physiology and biochemistry of the bacterial consortium in the granules had become preferentially acclimatized to life at an extreme thermophilic temperature.



**Fig. 5.** 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.

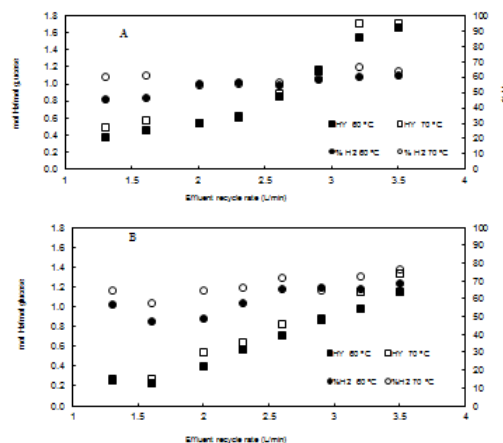
### 3.2. HYs at extreme thermophilic temperatures

In contrast to HP, HY followed the same trend as SHP by also decreasing with increasing biomass density (Fig. 6). 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<sub>2</sub>/mol glucose and 1.71 mol H<sub>2</sub>/mol glucose, at 60 °C and 70 °C, respectively (Fig. 6A). 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<sub>2</sub>/mol glucose and 1.34 mol H<sub>2</sub>/mol glucose, 60 °C and 70 °C, respectively (Fig. 6B). 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 °C resulted in a slight decrease in HYs at all degassed effluent recycle rates (Fig 6). The average HY (n=12) at 70 °C and 60 °C were 1.54 ± 0.29 mol H<sub>2</sub>/mol glucose and 1.34 ± 0.24 mol H<sub>2</sub>/mol glucose, respectively. For fluidized bed systems, reported mesophilic HYs range from 1.10 to 2.52 mol H<sub>2</sub>/mol glucose (Table 1). For a CSTR with a mixed bacterial floc culture an HY of 2.8 mol H<sub>2</sub>/mol glucose was achieved at mesophilic temperatures [17], ( see Table 1).

### 3.3. % H<sub>2</sub> and pH at hyperthermophilic temperatures

At 70 °C the % H<sub>2</sub> content ranged from 60 % to 77%, Whereas at 60 °C the % H<sub>2</sub> content fell between 60% and 65%, rarely rising above 70% (Fig. 6). It was also interesting to observe that when the pH of the effluent fell below 6.0 the % H<sub>2</sub> content also fell below 60%. In general the relationship between % H<sub>2</sub> content and pH shows considerable variability (Table 1). For example, at mesophilic temperatures (30 to 45 °C) the following % H<sub>2</sub> contents and corresponding pHs have been reported (Table 1): 72 % H<sub>2</sub> at pH 5.5 [17]; 59.2% H<sub>2</sub> at pH 5.5 [15]; 51% H<sub>2</sub> at pH 5.0 [10]; 37 % H<sub>2</sub> at pH 3.8 [9]; 32.6 to 41.7 % H<sub>2</sub> at pH 6.7 [2,3,16]; 42.3% H<sub>2</sub> at pH 6.2 [7]; 45% H<sub>2</sub> at pH 5.4 [4]. At thermophilic temperatures (50

to 60 °C) for high HP bioreactor processes the following trends have been reported (Table 1): 42 % H<sub>2</sub> at pH 5.0 [8]; 67 % H<sub>2</sub> at pH 5.5 [4].



**Fig. 6.** Influence of bacterial granular biomass density and effluent recycle rate on hydrogen yield and % H<sub>2</sub> content at extreme thermophilic and thermophilic temperatures. **A.** HY and % H<sub>2</sub> at influent rate of 6.3 L/h and bacterial biomass density of 6.3 g/L. **B.** HY and % H<sub>2</sub> at influent rate of 13.2 L/h and biomass density of 27 g/L.

## 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 1 gives an accurate summary of the current state of the art of high HP bioprocesses. What has become increasing clear is that % H<sub>2</sub> is highly variable for mixed bacterial cultures, ranging from 32.7 to 77 %. However, in our studies the % H<sub>2</sub> increased with increasing temperature, and in addition, the highest % H<sub>2</sub> contents were achieved at pHs that did not deviate far from 7.0.

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