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# Biohydrogen Production by Modified Anaerobic Fluidized Bed Reactor (AFBR) Using Mixed Bacterial Cultures in Thermophilic Condition

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## Abstract

Anaerobic fluidized bed reactor (AFBR) with slight modifications was investigated to increase biohydrogen production at high temperature. The modifications include a decrease in the total liquid volume to 3.3 L, in addition to an external work in the form of high temperatures, high dilution rates and high rates of de-gassed effluent recycling. These modifications were applied to overcome the thermodynamic constrains preventing the simultaneous achievement of high hydrogen yield (HY) and hydrogen productivity (HP) in an (AFBR). Bacterial granulation successfully induced under a high temperature of 65°C. The bacterial granules consisted of a multispecies bacterial consortium comprised of thermophilic clostridial and enterobacter species. Hydrogen production rate (HPR) of 7.57 L $H_2$/L/h and hydrogen yield of 5.82 mol $H_2$/ mol glucose were achieved at a hydraulic retention time (HRT) of 1 h and effluent recycle rate of 3.6 L/ min, with $V/F_{er}$ equal to 0.91

**Keywords:**
Dark fermentation,
High temperature,
Modified anaerobic fluidized bed reactor,
Biohydrogen production.

## 1. Introduction:

Biohydrogen production is the most challenging aspect with respect to environmental and renewable energy problems (Carere etal., 2008; Muradov & Vezirogu, 2008). Fermentative biohydrogen production yields energy and reduces waste. Biological $H_2$ production can performed by two methods: anaerobic fermentation and photosynthesis (Kotsopoulos et al., 2006). The efficiency of photosynthetic $H_2$ production is low and could not performed in the absence of light. (Levin et al., 2004) In contrast, fermentative $H_2$ can produce $H_2$ all day without light, using various kinds of substrates such as organic wastes, and has higher $H_2$ production rate, simple control requirements, lower operating costs and higher feasibility for industrialization (Li & Fang, 2007; Wang &Wang, 2009; Pugazhendhi et al., 2014). Thus, fermentative $H_2$ production is more feasible and widely used.

Dark fermentation using anaerobic fluidized bed reactor (AFBR) is promising and highly efficient in producing hydrogen gas in quantities exceeding even the theoretical values of 4 mol $H_2$/mol glucose if certain modification in the bioreactor design and process are made (Benemann, 1996; Levin et al., 2004; Van Ginkel & Logan, 2005). Currently, increasing efforts to improve hydrogen production by this promising approach are in doing. Although some of the research results are encouraging, there are still far way to go, with two hurdles need to addressed.

First, bioreactor designs require improvement in several important ways. Second, low cost raw materials needed to supply these reactors. As a corollary to both of these challenges, systems need to achieve higher levels of substrate conversion efficiency to reduce the product per-unit costs of both raw materials and processing. In the past five years, there have been many new developments in reactor optimization, raw material exploitation and two-stage hydrogen production (Ren et al., 2011). Under most bioreactor design and operating conditions, the maximum possible hydrogen yield (HY) in the anaerobic oxidation of glucose has generally been observed not to exceed 4 mol $H_2$/mol glucose (Das, 2009; Wang & Wang, 2009). Generally, (HY) did not to exceed or reach 70-100% of the maximum theoretical hydrogen yield. All the necessary and sufficient conditions that are most likely to facilitate the simultaneous achievement of high hydrogen yields and high hydrogen production may be reached by high microbial volumetric biomass density. This goal requires bacterial granulation, and high rate of microbial biomass retention within the bioreactor. The bacterial granules must have good settling properties, low hydraulic retention time or high dilution rate, and high organic substrate loading rate. Other fermentation conditions must be keep on as maintenance of the lowest possible partial $H_2$ pressure within the fluidized bacterial granular bed and efficient effluent gas-disengagement, high rates of de-gassed effluent recycling through the fluidized granular bed, maintenance of thermophilic temperatures within the bioreactor and minimization of dead volume within the bioreactor system. These conditions were the premises of the working hypothesis for the investigation undertaken in this study. Moreover, Space Time Yield (STYs) which represents the mass of a product P formed per volume of the reactor per time is low for biohydrogen production and this discouraged the production of hydrogen at commercial level. To overcome this constrains, it is essential to increase both HP and HY simultaneously. The STYs given by this equation: STY = mass of product P in kg / volume of the reactor in $m^3 \times$time in S. From the equation, it supposed that decreasing the total liquid volume of the AFBG system (V) relative to the degassed effluent recycle rate a simultaneous increase in both HP and HY can be achieved (Obazu et al., 2012).

## 2. Material and Methods:

### 2.1. Bioreactor nutrient medium formulation:

A modified Endo medium formulation (Noike & Matsumoto, 1982) used as the bioreactor influent medium in this study. The modification involved the reduction in the concentration of sodium bicarbonate from 6.72 g/L to 3.36 g/L. The inorganic minerals of the medium consisted (g/L) of: $NH_4HCO_3$ (3.490), $MnSO_4$ (0.015), $CaCl_2$ (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 $CoCl_2.H_2O$ ($1.24x10^{-4}$). The medium supplemented with 17.8 g sucrose/L (equivalent to 20 g COD/L) in distilled water.

### 2.2. Inoculum collection and preparation:

An anaerobic thermophilic bacterial consortium was derived from a mixture of sewage sludge and fresh wet goat manure. Sewage sludge was collected from an anaerobic sludge digester at Gaza municipal wastewater treatment works. Fresh goat dung was collected from grass-fed cattle at Al-Mughraqa, Middle Governorate, Gaza strip. Collected dung and sewage sludge samples were mixed in a 500 mL Schott bottles. The inoculum mixture was pre-conditioned by acid and heat-shock treated to enrich or select for anaerobic thermophilic hydrogen producing bacteria. Acid treatment performed by lowering the pH of the inoculum mixture to 2 with 1 M HCl and incubating for 24 h at room temperature to inhibit the activity of the methanogens. Following the acid treatment, the pH of inoculum mixture adjusted to 7.0 by mixing 50% v/v with Endo medium before heating at 90 °C in a water bath for 2 hours to remove non-sporulating hydrogen-consuming microorganisms, such as methanogenic microorganisms (Masilela, 2011). After the acid and heat treatments 250 mL samples of inoculum mixture was inoculated into 1 L Schott bottles containing 250 mL Endo medium and incubated at 65 °C in a shaking incubator set at 86 rpm. Cultures maintenance performed by sub-culturing into fresh Endo medium every 2 to 3 days (Masilela, 2011).

### 2.3. Bioreactor design and set-up:

Basically the modified bioreactor consists of a number of components (Figures 1 and 2):

**Figure 1** *Photograph of the experimental set up of the modified bioreactor*

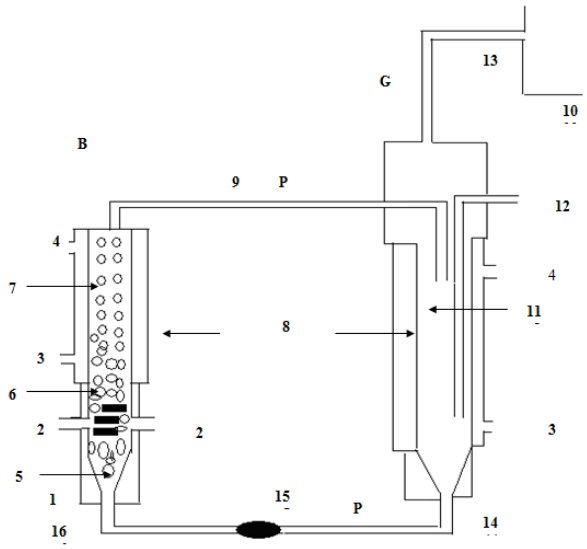


**Figure 2** *Modified AFGB system. Diagram labels:*
1- inlet manifold or diffuser; 2- influent inlets; 3- water jacket inlet for heat exchanger; 4- water jacket outlet for heat exchanger; 5- bed of glass beads in effluent/influent diffusion and for bubble generation through cavitation; 6- activated carbon for inducing granulation; 7- fluidized bacterial granular bed (B); 8- water jacket for heat exchanger; 9- effluent connecting pipe to gas disengager (P); 10- gas collector; 11- effluent gas disengager tube (G); 12- effluent outlet overflow pipe; 13- gas outflow pipe; 14-effluent recycle outlet pipe (P); 15- effluent recycle pump; and 16- effluent recycle inlet (P). Total AFGB volume: V= B+G+P.

The effluent gas disengager consists of a gas collection cylinder with height (H) =200mm and Internal Diameter (ID) =150mm connected to an effluent gas disengager cylinder (h = 545mm and ID= 60 mm). The effluent gas disengager has two effluent outlets one at the bottom that is connected to a variable peristaltic pump (0.4 kW, WG 600S +YZ 35 peristaltic pump from Xi an and Heb Biotechnology Co., Ltd. China) which is used to recycle degassed effluent into the bioreactor via the diffuser. The second effluent outlet drains the excess effluent overflow from the gas disengager. The liquid-gas separator or effluent gas disengager has a working volume of 1.54 L. At the base of the bioreactor (B) the stainlesssteel cylinder is connected to a conical shaped diffuser (h =150 mm and ID =80mm) made from stainless steel which functioned as the primary inlet for the effluent recycle stream. A stainless steel sieve (32 mesh) is fixed over the inlet of the diffuser. Above the stainless steel sieve the conical diffuser is filled with a 100 mm layer of 5 mm glass beads. Positioned at the upper end of the diffuser will be 4 inlet ports (ID =5mm) with each inlet arranged at 90° with respect to the two other inlets on each side. Nutrient medium (influent stream) is supplied directly into the upper glass bead layer via the 4 inlet ports. Bioreactor and gas disengager temperatures was maintained at the various operational temperatures (65°C) by circulating heated water from a heated water bath through the bioreactor and gas disengager water jackets. A variable peristaltic pump (BT/F 100F +YZ15 peristaltic pump from Xi an and Heb Biotechnology Co., Ltd. China), with a power less than 40W was used to pump the Endo nutrient into the bioreactor.

### 2.4. Operation strategy:

The operational strategy was carried out according to Masilela, P. (2011). On top of the glass bead, a 100 mm bed of charcoal (CAC) particles (diameter = 2.5 mm and length =5.0mm) was used to facilitate the induction of bacterial granulation in the bioreactor. 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. Sufficient CAC was added to the bioreactor to give a settled bed height of 100 mm. Endo medium 2 x (0.75 L) and treated seed inoculum (0.25 L) were added to the bioreactor system. Following inoculation, the bioreactor was operated on a batch effluent-recycle mode for 48 h at 65°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 HRT of 10 h. The HRT then gradually decreased over 2 day intervals by increasing the nutrient medium supply rate of 3.3 L/h. With further decreases in the HRT below 4h, the biofilm growth increased and bacterial granules began to form and accumulate at the surface of the expanded CAC bed. Once granule formation has initiated, further reductions in the HRT between 2 h and 1 h resulted in the rapid growth and expansion of the granular bed. Granule induction, growth and development were carried out at 65°C. The HRT was then gradually decreased over 2 day intervals by increasing the nutrient medium supply to a final rate of 3.3 L/h. The gas disengager has three out let: one at the end which is connected to the pump and used for effluent recycle and the other is on the top and connected to 12 L calibrated cylinder filled with water and used to measure the total gas produced by water displacement method. The third inlet is used to collect the excess over flow.

#### 2.4.1. Analytical techniques:

Gas analysis was performed by volumetric method. Twenty mL of the collected gas was taken by a syringe from the top of the gas collecting cylinder that has rubber cover and the gas was injected through a series of scotch bottles tightly closed. The first bottle contains 10% NaOH to precipitate $CO_2$ gas and the other contains 5% lead acetate to precipitate $H_2S$. The third bottle contains $H_2O$ which is collected in measuring cylinder upon displacement and accounted for $H_2$ gas. A standard with pure hydrogen gas was made to account for errors.

The following formula was used for converting total bioreactor gas flux (L/h) to mmol:

$$\frac{\Delta H_2}{\Delta t} = \frac{p_a\left[\%H_2{}^{vm}G_T\right]}{RT_a}$$

where, $\Delta H_2/\Delta t$ is mmol $H_2$/h; $P_a$ is the atmospheric pressure (101.3 kPa "Gaza area"); $\%H_2{}^{vm}$ is the percentage of hydrogen content from volumetric measurements (vm); $G_T$ (L/h) 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 monitored temperature at which the gas flow from the gas meter) (Noike & Matsumoto, 1982; Milne et al., 2002; Msilela, 2011; Obazu et al, 2012).

#### 2.4.2. Volatile Fatty Acids Analysis (VFAs):

A modified HPLC method after Koo-Ho Kwon et. al., (2014) used for Detection of VFAs (acetate, propionate, and butyrate) produced during fermentation in the bioreactor. Analysis of samples was performed by HPLC chromatography (Chrom Tech) using the AQUASIL C18, 5 µm, 250 x 4.6 mm column; eluent: 1% acetonitrile, 99% 0.05M $KH_2PO_4$; pH = 2.8; Flowing Rate (FR) = 1.25 mL/min and UV detection at 210 nm. Before performing any liquid measurements, samples subjected to filtration using a 0.22 µm membrane (Thermo scientific).

#### 2.4.3. Determination of Sucrose:

The concentration of sucrose in the reactor effluent and feed measured colorimetrically using the sucrose-resorcinol method. A solution of resorcinol reagent was prepared by dissolving 0.1 g resorcinol in 100 mL of 95% ethanol and 30% HCl was also made. A sucrose stock solution was prepared by dissolving 17 g of commercial sucrose into 1 L distilled $H_2O$. Thereafter, sucrose standard curves were then generated by mixing a known dilution of this standard (sucrose standard solution) with distilled $H_2O$ to a total volume of 1 mL in 10 mL test tubes. For the sucrose colorimetric assay, each sucrose standard curve dilution (1 mL) is mixed with 0.75 mL of 30% HCl and 0.75 mL of the resorcinol reagent and then incubated in at 80°C for 8 min, after which 2 mL distilled $H_2O$ was added to the sample. A spectrophotometer set at 520 nm was used for sucrose measurement against blank made with distilled $H_2O$ water as reference. Before performing colorimetric sucrose test, bioreactor effluent samples was subjected to a filtration using 0.22 µm membrane filters, then 1 mL of sample was used for sucrose determination according to above described method (Noike & Matsumoto, 1982; Milne et al., 2002; Masilela, 2011).

#### 2.4.4. Total bioreactor bacterial biomass determination:

The total biomass concentration in the reactor was determined gravimetrically. Upon opening the bioreactor at the end of experiment, the bioreactor content of suspension with bacterial cell was removed from the bioreactor and passed through 0.22 µm membrane filters. The residue collected on the filter dried in an oven at 65 °C for 48 hours. Thereafter the filter weighed after dried to determine the total biomass within the bioreactor (Noike & Matsumoto, 1982; Milne et al., 2002; Msilela, 2011).

#### 2.4.5. Microbial composition analysis:

A 10 ml sample suspended with granules withdrawn from the reactor. Portions of the sample were streaked on Macconkey agar plates to detect gram negative *Enterobacteriaceae*. Plates were incubated aerobically at 37ºC for 24 hours, and examined for colonial morphology. Other portions were streaked on pre-reduced oxygen blood agar plates and incubated under anaerobic conditions for 48 hrs to detect *Clostridium species*. Detection of *Clostridium species* confirmed by staining techniques and biochemical tests described by Bergey's Manual of Systematic Bacteriology (Vos D.P. et. al., 2009).

## 3. Results:

### 3.1. Bioreactor Setup and Design:

In this study, number of modifications were implemented in the bioreactor design and operation strategy, from old anaerobic fluidized bed bioreactor (AFBR) prototype (Wits bioreactor prototype) to overcome the thermodynamic constraints preventing the simultaneous attainment of both high production and high hydrogen yield by combination of external parameters such as high temperature, low HRTs and high recycles of de-gassed effluent. Firstly, the total volume of the original bioreactor system was reduced substantially to 3.3L. Secondly, operating the bioreactor at increasing effluent recycle rate gradually from 0.4 L/min to 3.6 L/min, while the effluent recycle rate was maintained at 3.6 L/min with simultaneous increases in the influent dilution rate or reduction in hydraulic retention time (HRT). Thirdly**,** the gas disengager was designed to assist in the reduction of the hydrogen concentration trapped in the liquid bulk phase. This was accomplished by facilitating stripping of the $H_2$ from the liquid phase within the gas disengager to the vapor phase, which was being continuously exhausted from the gas disengager. The mentioned modifications aimed to improve the ability of the reactor to reduce the hydrogen partial pressure within the reactor. As an overall result hydrogen production rate and hydrogen yield were improved, see Table 1.

### 3.2. Granule growth and microbial composition:

Following the inoculation of the bioreactors, granule formation is expected to take place within 3 days after the Endo medium supply rate or influent rate had reached 0.33 L/h (HRT of 10 h) when hydrogen gas can be detected. To grow the granular bed, the influent rate was then increased every two days. By the end of the run (24 days), the settled bed had grown to occupy the full size of the bioreactor (1L) and that was accompanied by increasing in hydrogen production and yield. This corresponded to a total granule dry mass of 7.41 g/L. Upon opening the bioreactor, the granules were almost filling the bioreactor. It was white in color indicating the absence of hydrogen sulfide and it had different shapes: spherical, epileptical, and hard mature granules. The bacteria on the granules showed predominant rod shaped bacteria. The bacteria on the granules showed predominant rod shaped bacteria. It also cultured under aerobic and anaerobic conditions. The spores of *Clostridium* species after 7 days culture under anaerobic conditions were dominant.

**Figure 3** *Sample culture from the AFBR reactor: (right) rod-shaped cells, (middle) hemolysis on blood agar under anaerobic conditions, (left) spore forming Clostridum*

### 3.3. Thermophilic bioreactor performance:

#### 3.3.1. Effect of hydraulic retention time (HRT) and effluent recycle rate on hydrogen production rate and hydrogen yield:

Both hydrogen production rate (HPR) and hydrogen yield (HY) versus HRT are shown in Figures 4 and 5 respectively. Hydrogen production was detected after 3 days (at HRT 10 h), as the HRT was gradually decreased from 10 h to1 h the hydrogen production rate (HPR) and hydrogen yield (HY) increased from 0.595 L $H_2$ /L/h and 2.904 mol $H_2$/mol glucose to 7.57 L$H_2$/L/h and 5.8 mol $H_2$/mol glucose, respectively, see Table 1. It also shown that as the effluent recycle rate increased gradually from 0.4 L/min to 3.6 L/min and HRT decreased to 1h, hydrogen production rate increased to 7.57 L $H_2$/L/h, while hydrogen content of raw gas was 69%.

The maximum hydrogen productivity of 310 mmol $H_2$/h was achieved at 1 h HRT with 3.3 L/h effluent feeding rate and a recycle rate of 3.6 L/min (Table 1). The experimental results showed that both hydrogen

production rate (HPR) and hydrogen yield (HY) increased significantly with the shortened HRT, giving the maximum at the shortest HRT of 1h of 7.57 L $H_2$/L/h and 5.8 mol H/mol glucose equivalent, respectively.

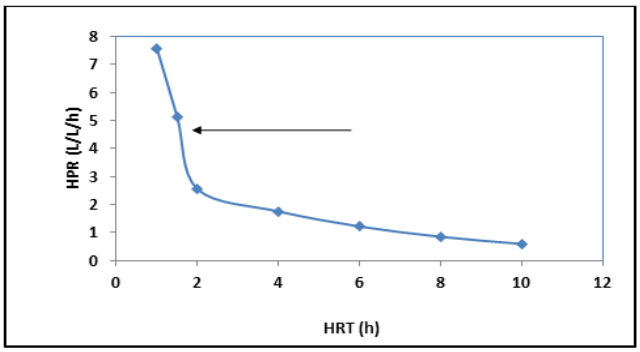


**Figure 4** *Hydrogen production rate (HPR) versus hydraulic retention time HRT*

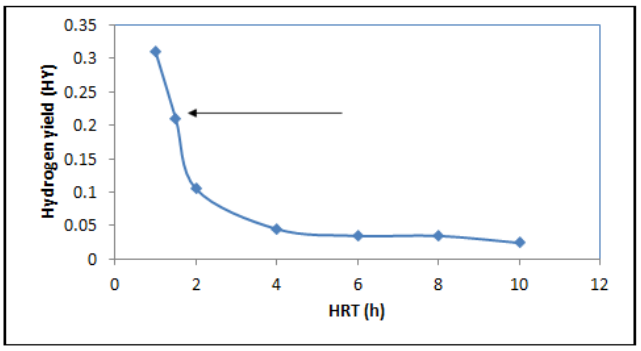


**Figure 5** *Hydrogen yield (HY) in mol/h versus HRT*

**Table 1** *Thermophilic bioreactor performance with respect to hydrogen production rate (HPR), hydrogen Productivity (HP) and hydrogen yield (HY), during 24 days of operation*

| Day | Actions taken | HRT (h). feeding rate (L/h) | Effluent recycle rate (L/min) | Rate of raw gas produced (L/h) | pH | Hydrogen content in raw gas % | Volumetric HPR (L $H_2$/L/h) | HP (mol $H_2$/L/h) | HY mol $H_2$ /mol glucose |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Sewage and dung were brought, mixed in scotch bottle. | - | | | 2 | | | | |
| 2 | pH of the Inoculum was raised to 7.2 using Endo Media | | | | | | | | |
| 3 | The system was operated in a batch manner. The column was fed with the Inoculum and 3x endo media | | 0.4 L/min | 1L/h | | No hydrogen was detected | | | |
| 4-6 | The system was shift to continuous process. | 10 h 0.33 L/h | 0.4 L/min | 1.7L/h | 3.3 | 35% | 0.595 L/h | 0.0244 | 2.904 |
| 7-9 | HRT decreased, recycle rate increased. | 8 h 0.4 | 1.5 L/min | 2.3 | 4.7 | 37 | 0.851 | 0.0348 | 3.844 |
| 10-12 | HRT decreased, recycle rate increased | 6h 0.55 | 2 L/min | 2.9 | 5.7 | 42 | 1.218 | 0.0450 | 3.98 |
| 13-15 | HRT decreased, recycle rate increased | 4h 0.825 | 2.5L /min | 3.9 | 5.9 | 45 | 1.755 | 0.0720 | 4.79 |
| 16-18 | HRT decreased, recycle rate increased | 2h 1.1 | 3L/min | 5.1 | 6.2 | 50 | 2.55 | 0.105 | 5.398 |
| 19-21 | HRT decreased, recycle rate increased | 1.5 h 2.2 | 3.4L/min | 8.1 | 7.3 | 63 | 5.122 | 0.2100 | 5.648 |
| 22-24 | HRT decreased, recycle rate increased | 1h 3.3 | 3.6 L/min | 10.98 | 7.8 | 69 | 7.57 | 0.310 | 5.827 |

* The experiment was conducted on summer June , August 2013.

### 3.3.2. Sucrose conversion rate versus HRT:

The sucrose conversion rate decreased apparently, from 98 % at 10 h to 62% at 1 h HRT (Tables 2&3). Regardless of decrease in sucrose conversion efficiency; the production of hydrogen increased and hydrogen ratio within the raw gas increased with the reduction in HRTs.

**Table 2** *Thermophilic bioreactor performance with respect to: Sucrose conversion rate, distribution of soluble metabolites, during 24 days of operation*

| HRT (h) /feeding rate (L/h) | Effluent recycle rate L/min | Concentration of sucrose in the effluent mmol/L | Concentration of acetate in the effluent mmol/L | Concentration of butyrate in the effluent mmol/L | Concentration of propionate in the effluent mmol/L | HP mol/L/h | HY mol $H_2$ /mol glucose |
|---|---|---|---|---|---|---|---|
| 10 h | | | | | | | |
| 0.33 | 0.4 | 3.6 | 4.39 | 4.85 | 8.34 | 0.0244 | 2.904 |
| 8 h | | | | | | | |
| 0.41 | 0.4 | 8.79 | 3.406 | 1.96 | 8.14 | 0.0348 | 3.8443 |
| 6h | | | | | | | |
| 0.55 | 1.5 | 9.96 | 3.3 | 2.12 | 5.36 | 0.0450 | 3.98 |
| 4h | | | | | | | |
| 0.825 | 2 | 12.036 | - | - | - | 0.0720 | 4.79 |
| 2h | | | | | | | |
| 1.1 | 2.5 | 12.27 | 2.8 | 2.13 | 3.65 | 105 0. | 5.398 |
| 1.5 h | | | | | | | |
| 2.2 | 3 | 13.88 | 2.49 | 1.70 | 2.48 | 0.2100 | 5.648 |
| 1h | | | | | | | |
| 3.3 | 3.6 | 14.6 | 2.29 | 0.48 | 1.75 | 0.310 | 5.827 |

**Table 3** *Thermophilic bioreactor performance with respect to sucrose conversion*

| Sucrose concentration in the feed (mM) | Sucrose concentration in the effluent (mM) | Sucrose utilized (mM) | % converted to $H_2$ | % Conversion rate |
|---|---|---|---|---|
| 51.65 | 3.6 | 48.05 | 35 | 98 |
| 57.76 | 8.79 | 49.00 | 37 | 85 |
| 61.03 | 9.96 | 51.34 | 42 | 79 |
| 78.73 | 12.00 | 66.73 | 45 | 70 |
| 89.99 | 12.2 | 77.79 | 50 | 68 |
| 131.9 | 13.88 | 118.02 | 63 | 65 |
| 168.79 | 14.6 | 154.37 | 69 | 62 |

### 3.3.3. Effect of pH change:

It is obvious that pH of the effluent increases by decreasing HRT and increasing recycle rate of effluent which accompanied by increasing of hydrogen production (HP) (Table 1). The pH values of effluent increased by increasing effluent recycle rate (Figure 6). The results shows that increasing of pH is accompanied by increasing of hydrogen production rate (HPR) up to 7.57 L $H_2$/L/h at pH 7.8, (Figure 7).

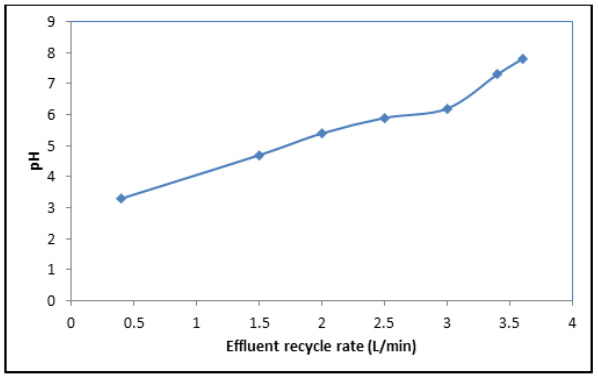


**Figure 6** *pH change versus effluent recycle rate*

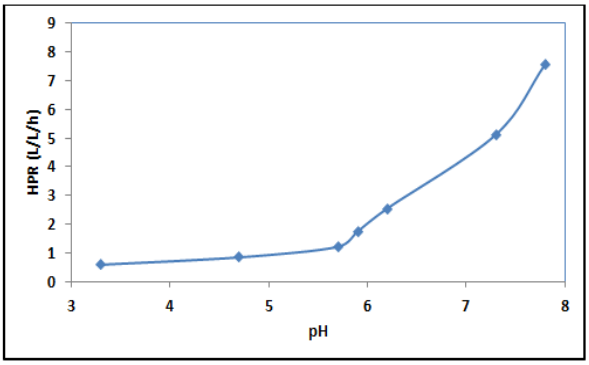


**Figure 7** *Hydrogen production rate (HPR) versus pH*

### 3.3.4. Effect of bioreactor working volume:

The substantial decrease in the bioreactor working volume to degassed effluent recycle ratio $V/F_{er}$ leads to increase in HY. The $V/F_{er}$ of different bioreactors volumes from other related similar studies compared with this study shown in Table 4.

**Table 4** *Thermophilic bioreactor performance with respect to total bioreactor volume and recycle rate*

| Bioreactor volume (B) L | Total volume (V) L | Effluent recycle rate L/min | Total volume: effluent recycle rate ratio $V/F_{er}$ (min | $H_2$ yield mol/mol | References |
|---|---|---|---|---|---|
| 5 | 12.6 | 3.5 | 3.61 | 1.84 | Obazu et al., 2012 |
| 3.27 | 10.5 | 3.5 | 3 | 2.84 | Obazu et al., 2012 |
| 5.072 | 7.5 | 3.5 | 2.14 | 3.905 | Masilela, 2011 |
| 3.27 | 5.74 | 3.2 | 1.79 | 3.55 | Obazu et al., 2012 |
| 1 | 3.3 | 3.6 | 0.91 | 5.8 | The present study |

## 4. Discussion:

Acid and heat pretreatment in this study was aiming to inhibit the activity of methanogenic bacteria in the anaerobic sludge. The effectiveness of the pretreatment was evident by the predominance of *Clostridia spp.* in the bed granules. This treatment ensures that the only evolved gases are hydrogen and carbon dioxide. Such treatment used to profiling the community composition of the microflora in activated sludge giving that Clostridium species in heat-treated activated sludge were the most commonly identified bacteria responsible for hydrogen production (Wang et al., 2007).

Many studies have shown that high hydrogen production rates achieved through improved bioreactor configurations (Chang et al., 2002; Chen et al., 2002; Kim et al, 2006; Li & Fang, 2007; Zhang et al., 2007).

In this study, it is succeeded to overcome the thermodynamic constraints preventing the simultaneous attainment of both high HPs and high HYs by combination of external parameters such as thermophilic temperature, low HRTs and high recycles of de-gassed effluent. Carrier induced thermophilic bacterial granulation has proven to be helpful in enhancing $H_2$ yield and providing stability to the process. The granules were suspected to be formed within a period of 5 to 9 days, and during days up to 24, in response to an influent rate of 3.3 L/h and effluent recycle rates of 3.6 L/min the fluidized granule bed occupied the full bioreactor working volume of 1 L, giving a fluidized bacterial dry mass density of 7.41 g /L.

The low solubility and low mass transfer coefficients of gases like hydrogen can delay the attainment of thermodynamic equilibrium between the different phases of the AFGB system. Gaseous fluxes from the AFGB system involves hydrogen mass transfer in different states of motion (Obazu et al, 2012).

In this study, it was proposed that high rates of effluent recycling achieved two essential process goals, which were necessary for increasing hydrogen yield. The first process goal was the rapid physical removal of $H_2$ trapped within fluid phase surrounding the bioreactor fluidized granular bed. The second process goal was the efficient effluent gas disengagement brought about by discharging the effluent at a high flow velocity from the bioreactor into the gas disengager tube. For effluent gas disengagement to be efficient, it would have been necessary for the process to remove most of the supersaturated concentration of dissolved hydrogen from the effluent before it was recycled back into the bioreactor. The $H_2$ content of the effluent recycled back into the bioreactor would correspond to the thermodynamic equilibrium dissolved hydrogen concentration, which would have been impossible to remove completely from the effluent stream in the gas disengager. So it was assumed that the effluent recycled back into the bioreactor would contain the thermodynamic equilibrium concentration of dissolved hydrogen. In about one minute, the entire fluid volume supporting the fluidized granular bed was displaced from the bioreactor with fresh degassed effluent mixed with influent nutrients, while the granular bed remained behind in the bioreactor. Recycling of degassed effluent at high velocity though the fluidized granules brings about the continuous and rapid displacement of $H_2$ containing bubbles and dissolved $H_2$ from the bioreactor bed. Thus the physical rate of the removal of $H_2$ trapped in the liquid phase was considerably greater than the rate at which $H_2$ was being generated by the granules embedded in the liquid

phase. An increase in temperature inhibits the $H_2$ consuming hydrogenases, thereby increasing the net flux of $H_2$ from the granules into the mobile fluid phase (Ngoma et al., 2011).

In addition, according to Le Chatelier's principle, under these operating conditions, the high rates of $H_2$ removal by the mobile fluid phase would make the $\Delta G^0$ more negative not only for the anaerobic oxidation of glucose, but also for the anaerobic oxidation of acetate, propionate and butyrate, in the absence of $H_2$ consuming bacteria. This would also add to the net flux of $H_2$ into the mobile liquid phase from the quasi-static fluidized granular bed. Thus the combination of high temperatures and a low $V/F_{er}$ quotient appears to be the necessary bioreactor operation conditions for the simultaneous achievement of high HPs and HYs.

Moreover, Space Time Yield (STYs) which represents the mass of a product P formed per volume of the reactor and time is low for biohydrogen production and this discouraged the production of hydrogen at commercial level. To overcome this constrain, it is essential to increase both HP and HY simultaneously (Obazu et al., 2012).

The STYs is given by this equation: STY = mass of product P in kg/ volume of the reactor in $m^3 \times$ time in S (Obazu et al., 2012).

The equation propose that by decreasing the total of liquid volume of the AFBG system (V) relative to the degassed effluent recycle rate a simultaneous increase in both HP and HY can be achieved (Gritzner & Kreysa, 1993). The total volume (V) of an AFGB system consists of the sum of the working bioreactor volume (B), the volume of the gas disengager (G) and finally the volume of the piping (P). This hypothesis would predict that for some critical value X, where $X = V/F_{er}$, HP will be some factor greater than120 mmole hydrogen/L/h, and HY will be equal to or greater than 3.0 mol $H_2$/mol glucose. A commercially viable STY 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. In this study the further reduction in the $X = V/F_{er}$ to 3.3/ 3.6 leads to increase in both hydrogen production and yield to (HP = 0.3100 mol $H_2$/L/h) and high hydrogen yields (HY = 5.8 mol /mol glucose).

It was suspected that the sharp increase in the effluent pH was as a result of low concentration of the acetate, propionate and butyrate in the bioreactor effluent. The effluent was mainly composed of acetate, propionate and butyrate. The findings showed that the concentration of acetate, propionate, and butyrate declined from 4.39 mM, 8.34 mM and 4.85 mM to 2.29 mM, 1.75 mM and 0.84 mM, respectively (Table 2).

With increasing temperature, concentrations of the volatile fatty acids,VFAs (acetate, butyrate and propionate) decreased. The rise in the HY and pH was consistent with decline in the concentration of the VFAs. Low HYs and high $H_2$ partial pressures are associated with the accumulation of the VFAs (acetate, propionate and butyrate). Under high $H_2$ partial pressures the aggregated reaction for the anaerobic oxidation of glucose by a multispecies bacterial consortium can be expressed as follows:

$$C_6H_{12}O_6 + tH_2O \rightarrow uCH_3COO^- + vCH_3CH_2COO^- + wCH_3CH_2CH_2COO^- + xH^+ + yHCO_3^- + zH_2.$$

With the different VFAs being produced in proportions corresponding to u:v:w, where u, v, and w are never zero. For the anaerobic oxidation of glucose, a decline in the values of u, v, and w for VFAs is consistent with a rise in the value of z for $H_2$. In this study, at low HRT (between 1.5 and 1 h) and with substrate concentrations equal to or greater than 10 g/L, the average concentration of acetate, propionate and butyrate were, 2.299 mM, 1.75 mM and 0.84 mM, respectively.

High butyrate and propionate concentrations were generally associated with low HYs. Increasing the HY above the theoretical threshold of 4.0 mol $H_2$/ mol glucose would require the anaerobic oxidation of acetate, butyrate and propionate in the absence of $H_2$ consuming bacteria. The anaerobic oxidation of the anions of alkanoic acids such as propionate and butyrate to acetate and $H_2$ becomes endergonic when the partial pressures of $H_2$ exceed 4Pa or the dissolved $H_2$ concentration exceeds 0.024 l M (Li & Fang, 2007). With a reduction in the $H_2$ partial pressure below 4 Pa, the oxidation of alkanoic acids and acetate becomes exergonic in the absence of $H_2$ consuming bacteria (Ngoma et al., 2011), and HYs should then exceed the theoretical limit of 4.0 mol $H_2$ /mol glucose.

A reduction in the $H_2$ partial pressures within the bioreactor to the levels necessary for achieving HYs equal to or greater than 4.0 mol $H_2$ /mol glucose represents a major challenge in the design and operation of AFGBs.

Another important factor in metabolic shifting is pH, the monitoring and control of the pH in a $H_2$ producing reactor is important not only for the control

of metabolic pathways (Lay, 2000), but also because pH serves as an inhibition mechanism for methanogens (Masilela, 2011). The choice of pH is important not only for the optimal production of $H_2$, but also for the production of volatile fatty acids (VFAs) and control of bacterial biomass growth. The accumulation of VFAs causes rapid drop in pH which is unfavourable to $H_2$ production. Moreover, some VFAs can be toxic or inhibitory to the $H_2$- producing microbial population (Masilela, 2011). As discussed by Van Ginkel et al., butyric acid could be more toxic than acetic acid in $H_2$ fermentation process, although there is no agreed threshold value for shifting from acidogenesis to solventogenesis (Van Gikle & Logan, 2005). The optimum pH reported for solventogenesis is around 4.5 while for acidogenesis, it is 5.5 or higher (Ferchichi et al., 2005). In this study, hydrogen production rate and yield increased with simultaneous increase in pH from 4.5 to 7.8, and after day 3, these observations were consistent with the decrease in all values of the concentration of VFAs. These results suggest that a change in pH value leads to the change in fatty acids concentration or composition thus driving more Nicotinamideadenine dinucleotide (NADH) for the formation of hydrogen. Importantly, a change of pH in fermentation system causes the shift of bacterial metabolites, and the carbon flux at high pH value has more trends to production of more acetate and eventually, results increased hydrogen production (Tang et al., 2008).

## Conclusion:

This study achieved simultaneously increase HY and HP when the bioreactor system and operational conditions had been modified in two significant ways: Firstly, the total volume of the bioreactor system relative the effluent volume recycle flux was reduced substantially. Secondly, following the reduction in the volume of the bioreactor system, the effluent recycle rate for a bioreactor with a working volume of 3.3 L was maintained at 3.6l / min and the dilution rate was increased. All these modifications resulted in an increase in the HY (4.7 mol $H_2$/mol glucose) confirming that high HYs and HPs could be simultaneously achieved. In addition, when the temperature was high at high influent flow rates and at high effluent recycles rates the following results were obtained:

(1 ) High HYs and HPs were simultaneously achieved.

(2) Volatile fatty acids (VFAs) such as acetate, butyrate and propionate decreased, indicating that at temperatures greater than $55^0$ C under these bioreactor operation conditions the oxidation of acetate, butyrate and propionate became thermodynamically favorable.

Carbon balance analysis in terms of sucrose concentration in influent and effluent streams confirmed that VFAs oxidation was taking place at temperatures greater than $55^0$C. Under these operational conditions VFAs were being oxidized to hydrogen. This result is consistent with further experimental results that gave HYs greater than 4 mol $H_2$/mol glucose.

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## إنتاج الهيدروجين الحيوي من مجموعة بكتيريا في ظروف لا هوائية وحرارة عالية بواسطة المفاعل اللاهوائي المميع والمكون للحبيبات

بحثت الدراسة زيادة إنتاج الهيدروجين الحيوي بواسطة تحوير المفاعل اللاهوائي المميع عند درجات الحرارة المرتفعة. شملت التحويرات على المفاعل إنقاص الحجم الكلي للمفاعل حتى 3.3 لتر بالإضافة إلى تطبيق شغل خارجي على شكل حرارة مرتفعة وكذلك معدل تخفيف مرتفع علاوة على معدل تدوير مرتفع من الدفق المخرجات المنزوعة الغاز. تم تطبيق التحويرات المذكورة بهدف التغلب على القيود الثيرموديناميكية التي تمنع تحقيق محصول مرتفع و إنتاجية مرتفعة من الهيدروجين في وقت واحد. تم بنجاح تحفيز تكوين حبيبات من البكتيريا تحت ظروف حرارة 65 درجة مئوية. وجد أن الحبيبات البكتيرية تحتوي على عدة أنواع من البكتيريا الأمعائية والكلوستريدية المحبة للحرارة مع سيادة في جنس الكلوستريديا. وصل معدل إنتاج الهيدروجين حتى 7.57 لتر هيدروجين لكل لتر من المفاعل في الساعة الواحدة كما تم تحقيق محصول من إنتاج الهيدروجين بمقدار 5.82 مول من الهيدروجين لكل مول من مكافئ الجلوكوز وذلك عند زمن مكوث هيدروليكي ساعة واحدة ومعدل تدوير لدفق المخرجات بمقدار 3.6 لتر في الدقيقة كما وصلت نسبة الحجم الفاعل للمخمر إلى حجم دفق المخرجات المعاد

**كلمات مفتاحية:**
التخمر في الظلام،
الحرارة المرتفعة،
مفاعل لاهوائي مميع محور،
الهيدروجين الحيوي.