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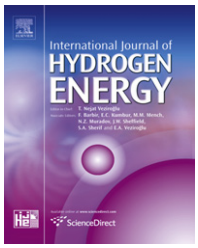

Simulating the impact of suppression of methanogenesis in continuous flow biohydrogen reactors

Hisham Hafez [a,*], Elsayed Elbeshbishy [b], George Nakhla [b,c], M. Hesham El. Naggar [b]

[a] GreenField Ethanol Inc., Chatham, Ontario, Canada N7M 5J4
[b] Department of Civil Engineering, The University of Western Ontario, London, Ontario, Canada N6A 5B9
[c] Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario, Canada N6A 5B9

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ABSTRACT

A calibrated model of the patent-pending integrated biohydrogen reactor clarifier system (IBRCS) using BioWin was used to evaluate the impact of sludge and/or feedstock pre-treatment for methanogens inhibition in a dynamic simulation for 90 days with and without methanogens suppression. Dynamic simulations at four different OLRs ranging from 6.5 to 103 gCOD/L-d have shown that without any pre-treatment, the system was capable of washing out methanogens and enriching hydrogen producers. The average methane gas content in the reactor's headspace was 4% after 7 days of continuous operation, decreasing sharply to less than 0.5%, while the maximum reduction in hydrogen gas was <10%. The hydrogen gas content in the headspace ranged from 65% to 72%. Simulating the impact of extended SRT ranging from 3 days to 20 days on the performance of the IBRCS revealed that up to an SRT of 5 days hydrogen production was predominant with a reasonable deterioration in the production rate by 20%. Biomass distribution showed that at SRTs up to 20 days, acetoclastic methanogens were naturally eliminated. However, hydrogenotrophic methanogens had a significant impact on the overall hydrogen production rate where most of the hydrogen gas produced was consumed at SRTs of 10 days and 20 days.

1. Introduction

Biological hydrogen production from renewable sources [1] has the potential to meet the growing demand for energy. It offers a feasible means for sustainable supply of H_2 with low pollution and high efficiency, thereby considered a promising eco-friendly energy source [2]. Comparing the production rates of H_2 by various biohydrogen systems and the associated operational complexity, confirms that dark fermentation systems offer an excellent potential for practical applications [3], and hence the great interest from the scientific community.

In dark fermentation, when mixed cultures are used, hydrogen-consuming bacteria (e.g. methanogens and homoacetogens) must be eliminated or inhibited to prevent hydrogen consumption [4–6]. When mixed cultures are treated under harsh conditions, hydrogen-producing bacteria have the ability to form spores which give them a better chance to survive than some non-spore forming hydrogen-consuming bacteria [7]. Thus, mixed cultures have to be pre-treated to suppress methanogens and hydrogen-consuming bacterial (e.g. methanogens and homoacetogens). Pre-treatment methods for enriching hydrogen-producing bacteria

* *Corresponding author*. Tel.: +1 519 784 6230; fax: +1 519 352 9559.
E-mail addresses: hishambahaa@gmail.com, h.hafez@greenfieldethanol.com (H. Hafez).
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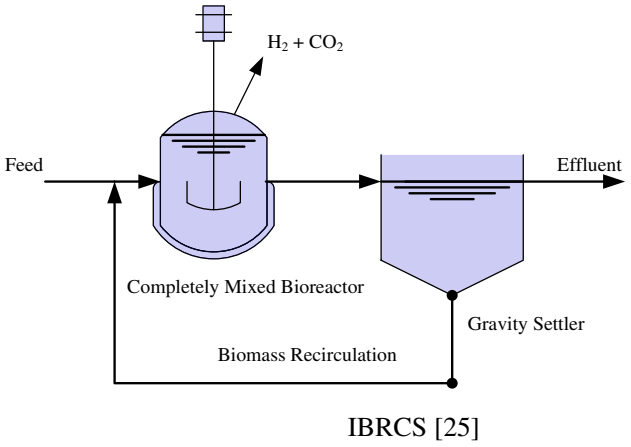

Fig. 1 – Experimental Set up for the integrated biohydrogen reactor clarifier system.

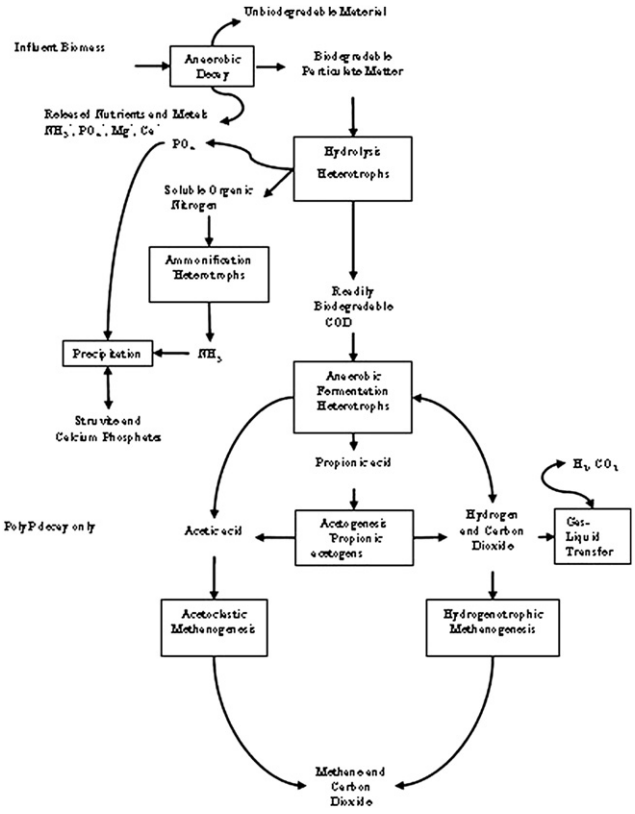

Fig. 2 – Conceptual schematic for the anaerobic degradation model in BioWin (Adapted from BioWin manual).

from mixed cultures mainly include [6] heat-shock (at relatively low temperatures of 75 °C and 85 °C [8,9] as well as relatively high temperature of 104 °C [10]), acid, base [11], aeration [12], freezing and thawing [1], chloroform [13], sodium 2-bromoethanesulfonate or 2-bromoethanesulfonic acid and iodopropane [12,14]. Pre-treatment methods are primarily judged based on their efficiency in eliminating methanogenic activity and enhancing hydrogen yield [6]. Even though heat-shock was the most widely used pre-treatment method for enriching hydrogen-producing bacteria from inocula [15], it is not always effective for enriching hydrogen-producing bacteria from mixed culture inocula compared with other pre-treatment methods, as it may inhibit the activity of some non-spore forming hydrogen-producing bacteria [11].

Numerous pure cultures of bacteria have been used to produce hydrogen from various substrates. The majority of studies involving anaerobic hydrogen production have involved the use of *Clostridium* bacteria; high yields have been obtained using inoculum of pure cultures, mixed anaerobic communities where *Clostridia* were shown to be the dominant organisms, as well as individual strains isolated from waste material [16–18]. Tests with pure bacterial cultures for fermentative hydrogen production were conducted in batches and used glucose as substrate [19–21]. However, continuous hydrogen production from organic waste is more feasible for industrialization to realize the goals of waste reduction and energy production [15]. The disadvantage of using a pure strain is that sterile feedstock conditions (free of methanogens and/or hydrogen-consuming bacteria) should be maintained throughout the process, which is impractical on a large industrial scale [22]. Various pre-treatment methods were applied on real feedstocks. Freezing and thawing and sterilization were superior pre-treatment methods for fermentative hydrogen production [23,24].

The aforementioned paragraph highlights the different methods of pre-treatment that were applied to either bacterial inoculum or feedstocks. Although, it appears that most of these methods are effective for methanogens suppression, on a large scale application where continuous hydrogen production will be used, they all seem impractical and economically unfeasible. Thus, in the present study a process model using BioWin (EnviroSim Associates LTD., Flamborough, Ontario, Canada) that was developed, calibrated, verified and presented in our earlier work [3], will be used to dynamically simulate and evaluate the impact of pre-treatment for methanogens suppression on a novel integrated biohydrogen reactor clarifier system (IBRCS) [25] (see Fig. 1). The system is comprised of a continuously stirred-tank reactor (CSTR) for biological hydrogen production, followed by an uncovered gravity settler for decoupling of solids retention time (SRT) from hydraulic retention time (HRT). In addition, the model will be used to define the maximum SRT for biohydrogen systems that maximizes process performance and

Table 1 – Operational conditions.

	Glucose (g/L)	HRT (h)	SRT (h)	OLR (gCOD/L-d)	Final pH
OLR-1	2	8	50 ± 5	6.5	5.5
OLR-2	8	8	45 ± 4	25.7	5.5
OLR-3	16	8	45 ± 6	51.4	5.5
OLR-4	32	8	42 ± 6	103	5.5
OLR-5	48	8	27 ± 3	154	5.5
OLR-6	64	8	26 ± 2	206	5.5

Note. Values represent average ± standard deviation.

Table 2 – Wastewater fractions.

Name	Value
Readily biodegradable [gCOD/g of total COD]	0.8
Acetate [gCOD/g of readily biodegradable COD]	0
Non-colloidal slowly biodegradable [gCOD/g of slowly degradable COD]	0.5
Unbiodegradable soluble [gCOD/g of total COD]	0.018
Unbiodegradable particulate [gCOD/g of total COD]	0.04
Ammonia [gNH3-N/gTKN]	0.5
Particulate organic nitrogen [gN/g Organic N]	0.25
Soluble unbiodegradable TKN [gN/gTKN]	0.02
N:COD ratio for unbiodegradable part. COD [gN/gCOD]	0.035
Phosphate [gPO4-P/gTP]	0.2
P:COD ratio for influent unbiodegradable part. COD [gP/gCOD]	0.011
Non-poly-P heterotrophs [gCOD/g of total COD]	1.00E-04
Anoxic methanol utilizers [gCOD/g of total COD]	1.00E-04
Ammonia oxidizers [gCOD/g of total COD]	1.00E-04
Nitrite oxidizers [gCOD/g of total COD]	1.00E-04
Anaerobic ammonia oxidizers [gCOD/g of total COD]	1.00E-04
PAOs [gCOD/g of total COD]	1.00E-04
Propionic acetogens [gCOD/g of total COD]	1.00E-04
Acetoclastic methanogens [gCOD/g of total COD]	1.00E-04
H_2-utilizing methanogens [gCOD/g of total COD]	1.00E-04

eliminates any methanogenic activity with a particular focus on biomass distribution.

2. Materials and methods

This section provides a brief description of the experimental set up, procedures and operational conditions at which the experimental data for the IBRCS, used to calibrate and validate the process, was collected. Additional information can be found in Hafez et al. [3].

2.1. Systems set up and operations

Two lab-scale IBRCSs were considered in the study, each comprising of a continuously stirred-tank reactor (CSTR) for biological hydrogen production (5 L working volume), followed by an uncovered gravity settler (volume 8 L) i.e. open to the atmosphere for the decoupling of solids retention time (SRT) from the hydraulic retention time (HRT). Both systems were operated at 37°°C for 220 days (Fig. 1), at six different organic loading rates (OLR) ranging from 6.5 to 206 gCOD/L-d. Details of the operational conditions for the six runs are listed in Table 1. It is noteworthy that the systems were run at steady state conditions for at least 20 turnovers of the mean SRT, with the shortest run lasting for 45 days and the longest run for 75 days, excluding the first week of startup. In order to enrich hydrogen-producing bacteria, the seed sludges were heat treated at 70°°C for 30 min prior to startup. Following the completion of each run and the attainment of steady state conditions, the systems were cleaned and inoculated with pre-treated sludges. OLR-1 and 2 were run simultaneously, followed by OLR-3 and 4, and lastly OLR-5 and 6. Details of analytical methods are reported elsewhere [26].

2.2. Inocula and media compositions

Anaerobically-digested sludge from the St. Marys wastewater treatment plant (St. Marys, Ontario, Canada) was used as the seed. The two systems operated in parallel at the same time under two different OLRs for a total of six OLRs (three consecutive runs). The systems were seeded with 5 L of sludge and started up in a continuous mode with the feed containing glucose at different concentrations as presented in Table 1. The same startup technique was repeated for the three runs. It must be emphasized that there was no sludge wastage from the clarifier throughout the operation, and the values of SRTs presented in Table 1 represent the average ± standard deviation (SD) during steady state operation. It is noteworthy that the operation of the reactors was consistent over time and accordingly, the average SRT with SD of less than 10% of the mean SRT is representative of the overall SRT during the run. The SRT was estimated according to the amount of VSS (g) in the hydrogen bioreactor (excluding biomass in the clarifier) divided by the amount of VSS (g/d) leaving the system in the clarifier liquid effluent. To evaluate the settling characteristics of the biomass, both zone settling velocity (ZSV) and sludge volume index (SVI) were performed on a weekly basis throughout the study. The ZSV ranged from 120 to 240 m/d and SVI from 82 to 110 mL/g. It is noteworthy that the zone settling velocity increased almost linearly with biomass average particle size up to 52 μm, and stabilized at around 240 m/d thereafter. Similarly SVI decreased linearly with particle size up to 52 μm and stabilized at around 80 mL/g thereafter. The relationship between the ZSV and SVI (not shown) was inversely linear with R^2 of 0.825. The settleability of the hydrogen producers was considered to be superior to activated sludge since SVI of 100 mL/g and ZSV of 100 m/d are considered typical for good settling activated sludge.

2.3. Model formulation

The anaerobic degradation processes in the BioWin model are based on the "four population" model concept (heterotrophs, acetogens, acetoclastic methanogenesis and hydrogenotrophic methanogenesis). A conceptual schematic of the biohydrogen production model is shown in Fig. 2. For detailed

Table 3 – Kinetic parameters for Heterotrophs.

Name	Default	Value	Arrhenius
Max. spec. growth rate [1/d]	3.2	3.2	1.029
Substrate half sat. [mgCOD/L]	5	5	1
anaerobic decay [1/d]	0.3	0.3	1.029
Anaerobic hydrolysis factor [-]	0.5	0.5	1
Adsorption rate of colloids [L/(mgCOD d)]	0.8	0.8	1.029
Fermentation rate [1/d]	3.2	3.2	1.029
Fermentation half sat. [mgCOD/L]	5	5	1
Hydrolysis half sat. [mgCOD/L]	0.15	0.15	1

Table 4 – Kinetic parameters for Methanogens.

Name	Default	Methanogens OFF	Methanogens-ON	Arrhenius
Acetoclastic Mu Max [1/d]	0.3	0	0.3	1.029
H_2-utilizing Mu Max [1/d]	1.4	0	1.4	1.029
Acetoclastic Ks [mgCOD/L]	100	100	100	1
H_2-utilizing CO_2 half sat. [mmol/L]	0.1	0.1	0.1	1
H_2-utilizing Ks [mgCOD/L]	0.1	0.1	0.1	1
Acetoclastic propionic inhibition [mgCOD/L]	10000	10000	10000	1
Acetoclastic decay rate [1/d]	0.13	0.13	0.13	1.029
H_2-utilizing decay rate [1/d]	0.13	0.13	0.13	1.029

description of the structure of the model please refer to Hafez et al. [3]. The influent characteristics of the synthetic wastewater containing glucose used in the experimental study was simulated in the model using the influent specifier associated with BioWin model and revealed the fractions summarized in Table 2. The main kinetic parameters for heterotrophs (hydrogen producers) used in all modeling runs were set to default values (see Table 3). To simulate the inhibition of methanogens due to pre-treatment of the seed sludge, the methanogens growth rates were switched off while in the unpre-treated seed sludge runs, the growth rates were maintained at the default value (see Table 4). In our previous work [3], the model accurately predicted biomass concentrations in both the bioreactor and the clarifier supernatant with average percentage errors (APE) of 4.6% and 10%, respectively. Hydrogen production rates and hydrogen yields predicted by the model were in close agreement with the observed experimental results as reflected by an APE of less than 4%, while the hydrogen content was well correlated with an APE 10%. The successful modeling culminated in the accurate prediction of soluble metabolites i.e. volatile fatty acids in the reactor with an APE of 14%.

3. Results and discussion

3.1. *Dynamic simulations at different OLRs*

Fig. 3 shows the diurnal variations in hydrogen and methane production rates for the dynamic simulations incorporating both suppressed and unsuppressed methanogens at OLRs ranging from 6.5 gCOD/L-d up to the optimum OLR of 103 gCOD/L-d. It is noteworthy that optimum OLR for hydrogen production in the IBRCS was defined in our earlier work [26]. As depicted in Fig. 3a, over a period of 7 days, which represents 3 turnovers of the system's SRT of 2.4 days, the hydrogen production rate in the methanogens OFF run, increased drastically to approximately 11.8 L/d reaching a plateau at 12.35 L/d after 2 weeks from startup and lasting for 90 days of continuous operation. On the other hand, there was no

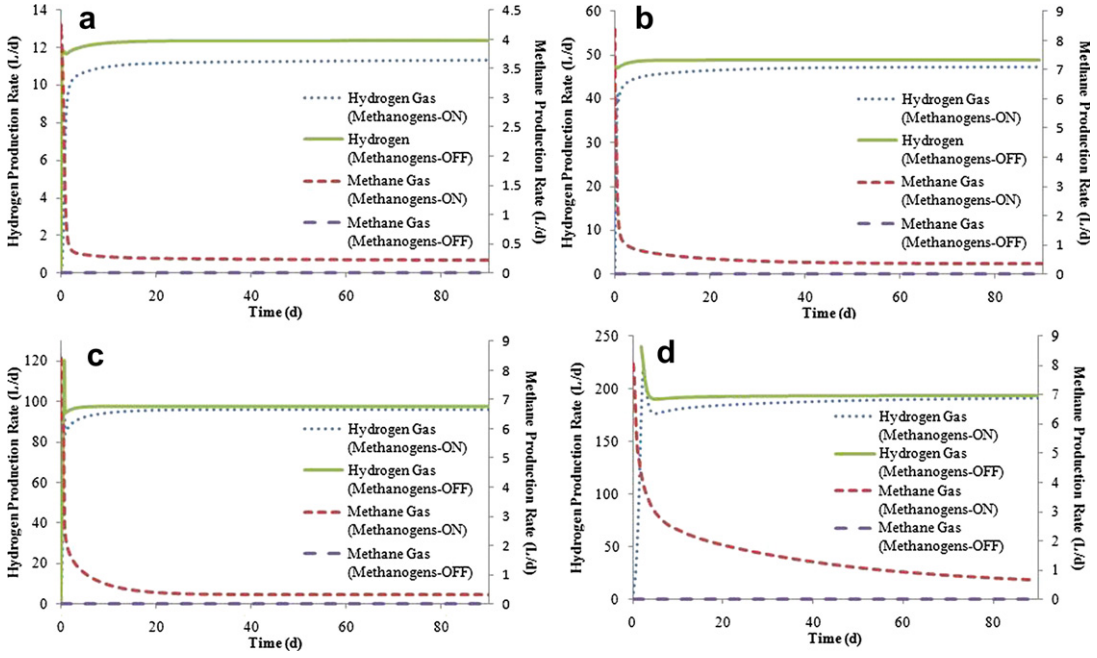

Fig. 3 – Dynamic simulations for hydrogen and methane production rates at: a) OLR-1 = 6.5 gCOD/L-d, b) OLR-2 = 25.7 gCOD/L-d, c) OLR-3 = 51.4 gCOD/L-d, d) OLR-4 = 103 gCOD/L-d.

Table 5 – Summary of dynamic simulations.

	OLR-1		OLR-2		OLR-3		OLR-4	
	ON	OFF	ON	OFF	ON	OFF	ON	OFF
Gas Flow Rate (L/d)	16 ± 1	17 ± 1	70 ± 2	73 ± 1	144 ± 7	146 ± 6	287 ± 27	291 ± 25
H_2 (%)	70 ± 5	72 ± 2	66 ± 2	67 ± 2	66 ± 2	67 ± 2	65 ± 3	66 ± 2
CH_4 (%)	2 ± 4	0	1 ± 2	0	0 ± 2	0	0.7 ± 1.5	0
VSS (mg/L)	1400 ± 500	1400 ± 500	4500 ± 320	4500 ± 300	9000 ± 180	9000 ± 180	15700 ± 1000	15700 ± 1000
VFAs (mg/L)	1600 ± 85	1600 ± 73	6400 ± 350	6400 ± 340	12800 ± 800	12800 ± 800	24500 ± 3400	24500 ± 3400
H_2 (L/d)	11 ± 1	12 ± 0.3	47 ± 2	49 ± 1.5	95 ± 6	97 ± 4	185 ± 19	192 ± 17
CH_4 (L/d)	0.3 ± 0.7	0	0.5 ± 0.7	0	0.5 ± 0.8	0	1.6 ± 1.2	0
SRT	2.4	2.4	1.9	1.9	1.9	1.9	1.6	1.6
Alkalinity (mg/L)	700	700	2900	2900	5700	5700	11450	11450
VFA/alkalinity ratio	2.3	2.3	2.2	2.2	2.2	2.2	2.1	2.1
pH	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.2	5.5 ± 0.2

Note: ON represents unsuppressed methanogens, OFF represents suppressed methanogens, values represent average ± standard deviation.

methane production as methanogens were already set to OFF as mentioned earlier. Similarly, at OLR-2, OLR-3 and OLR-4, hydrogen production rate followed the same trend (see Fig. 3b,c and d) where in 2 weeks after startup, a stable hydrogen production rate of 50 L/d, 100 L/d and 200 L/d was established, respectively. To test the impact of methanogens on the IBRCS, a set of dynamic simulations were performed over the same range of organic loading rates and with the exact operational conditions of the dynamic simulations "methanogens-OFF" except that the methanogens were switched to ON and their kinetic parameters were set to the default values (see Table 4). Fig. 3a shows that the methane production rate decreased drastically from 4.5 L/d to 0.8 L/d over a period of 10 days and stabilized at less than 0.5 L/d thereafter. While at OLR-2 to OLR-4, the methane production rate decreased from 8 L/d initially to less than 0.5 L/d for OLRs 2 and 3 and to less than 1 L/d at OLR-4 over a period of 25 days. On the other side, switching the methanogens to ON exerted an insignificant impact on hydrogen production at the 4 OLRs as evident from the maximum reduction in hydrogen production rate of 11% (refer to Table 5) that occurred at an OLR-1 (6.5 gCOD/L-d). The stability of hydrogen production in the IBRCS was clearly emphasized by comparing the main process parameters for the ON and OFF simulations. As shown in Table 5, the concentration of VSS, VFAs and the total amount of biogas produced were not affected by the

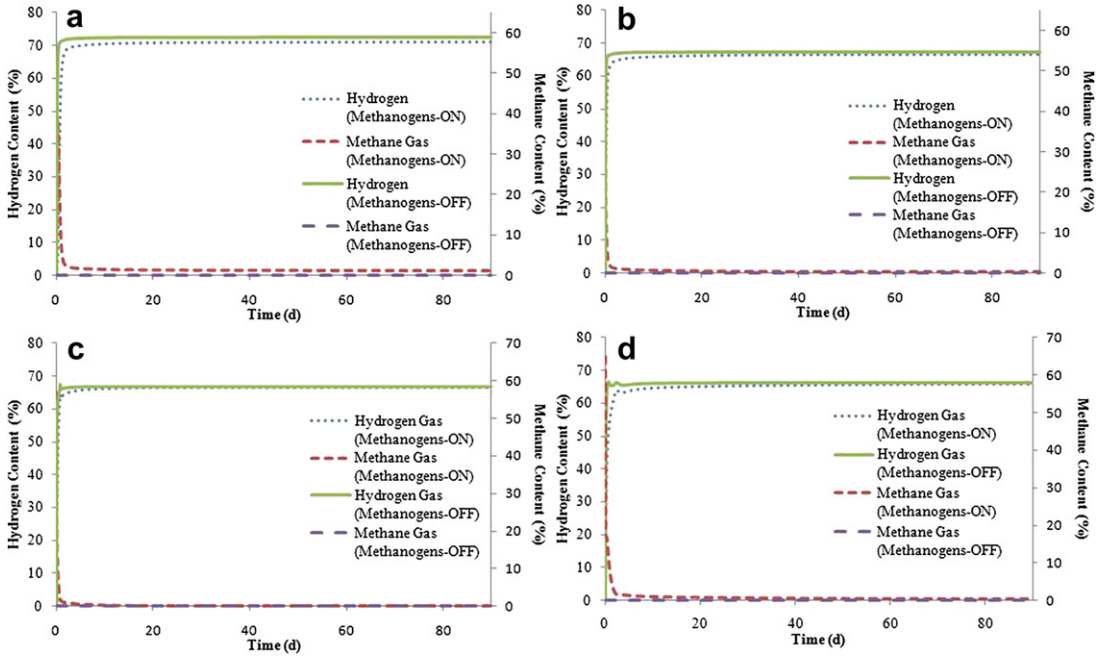

Fig. 4 – Dynamic simulations for biogas content at: a) OLR-1 = 6.5 gCOD/L-d, b) OLR-2 = 25.7 gCOD/L-d, c) OLR-3 = 51.4 gCOD/L-d, d) OLR-1 = 103 gCOD/L-d.

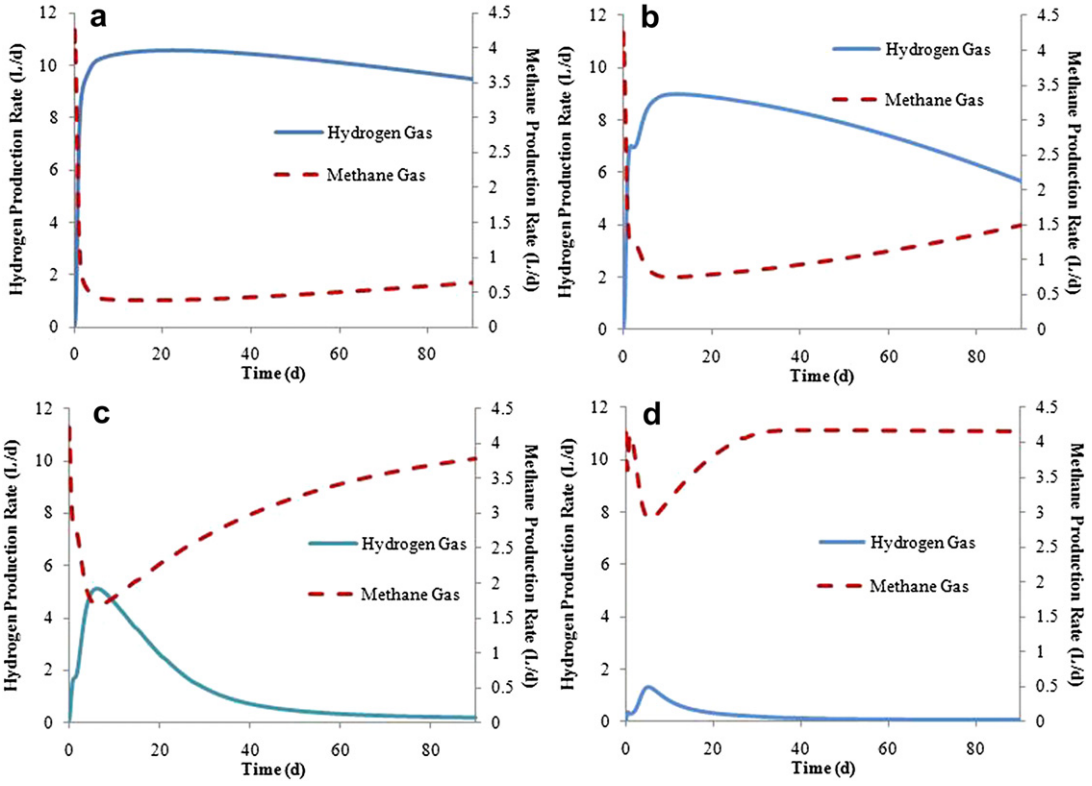

Fig. 5 – Diurnal variations in biogas production rate at OLR-1 = 6.5 gCOD/L-d and methanogens-ON for a) SRT = 3 days, b) SRT = 5 days, c) SRT = 10 days, d) SRT = 20 days.

methanogenic culture. Fig. 4 shows the diurnal variation in biogas quality in the headspace of the IBRCS at the 4 OLRs. The maximum methane content in the biogas was 6% at the lowest OLR (6.5 gCOD/L-d). The average hydrogen content in the headspace ranged from 65% to 72%.

Fortunately, most of the operational conditions that favor continuous hydrogen production in dark fermentation are extremely unfavorable for methanogens. To rationalize the insignificant impact of the pre-treatment of seed sludges on the IBRCS, four important parameters were evaluated i.e. maximum specific growth rate (μ_{max}), HRT and SRT, operational pH, and VFA to alkalinity ratio. The VFA to alkalinity ratio and pH are crucial parameters in both anaerobic digestion (for methane production) and dark fermentation (for hydrogen production). The optimum range of VFA to alkalinity ratio for methane production in anaerobic digesters ranges from 0.3 to 0.4 to prevent process failure [27] while a VFA to alkalinity ratio in the range of 1.5–2.5 is required for a stable hydrogen production in dark fermentation [28]. Moreover, Acharya and Kurian [29] reported a complete failure in their anaerobic digester at a VFA to alkalinity ratio close to 2.5 and a pH of less than 6. As summarized in Table 5, the average VFA to alkalinity ratio in all simulation runs was around 2.2 and the reactor's pH was around 5.5.

The default maximum specific growth rates (μ_{max}) for hydrogen producers, acetoclastic methanogens, and hydrogenotrophic (hydrogen utilizing) methanogens in BioWin are 3.2 d^{-1}, 0.3 d^{-1}, and 1.4 d^{-1}, respectively. It is clear that the acetoclastic methanogens growth rate is 1/10 of that of hydrogen producers corresponding to a minimum HRT or SRT (for decoupling systems) of 3.5 days which is greater than the maximum SRT of 2.4 days reported in Table 5 and in our earlier experimental work [26]. On the other hand, the μ_{max} of hydrogenotrophic methanogens is only 50% of that of hydrogen producers corresponding to SRT_{min} of 0.7 day. Applying a safety factor of 6 [30], the design SRT (SRT_{design}) for the overall methanogenic community, as to cease washout, should not be less than 4.2 days (4.2 days for hydrogenotrophic methanogens and 20 days for acetoclastic methanogens) at normal environmental conditions (appropriate: VFA/alkalinity ratio, pH and OLR) as μ_{max} drops drastically by at least 50% at harsh conditions [30]. Thus, it is clearly evident that the short HRT of 8 h and SRT of 2.4 days facilitated the washout of the methanogens. Having said this, simulation runs with extended SRTs of 3, 5, 10 and 20 days are presented below.

3.2. *Dynamic simulations at different SRTs*

In our earlier work [26], biomass concentration in hydrogen reactors was proven to be a key parameter for the stability of hydrogen production as it directly affects the food-to-microorganisms (F/M) ratio. Both molar hydrogen yield and biomass specific hydrogen production rate were found to drop precipitously at F/M ratio above 6.4 gCOD/gVSS-d. In the literature, biohydrogen system failures were frequently attributed to marked decrease in biomass content in the hydrogen reactor due to severe cell washout [31–33]. Thus, in systems that

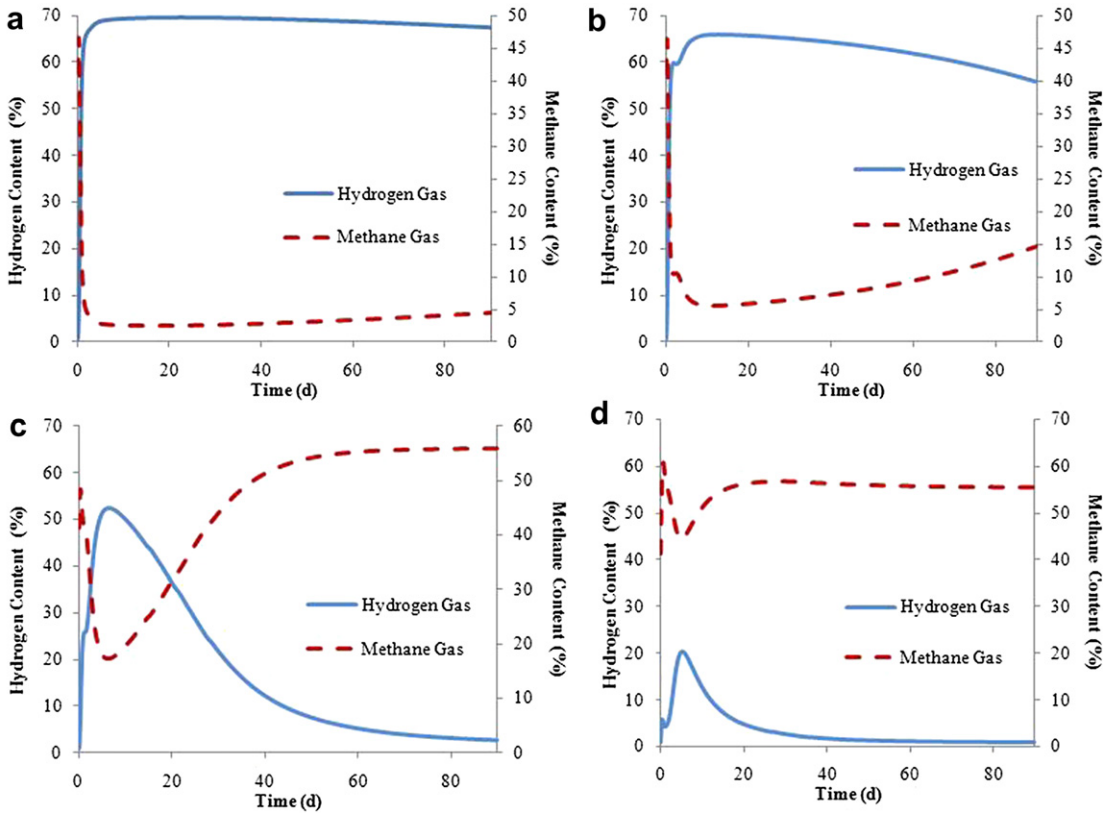

Fig. 6 – Diurnal variations in biogas content at OLR-1 = 6.5 gCOD/L-d and methanogens-ON for a) SRT = 3 days, b) SRT = 5 days, c) SRT = 10 days, d) SRT = 20 days.

decouple SRT from the HRT and optimized for hydrogen production where F/M ratio is crucial, it is important to define the maximum SRT the system can operate at without detection of and/or with minimal methanogenic activity. Operating at longer SRTs will facilitate running the process at higher biomass concentrations and will decrease biomass yield leading to a significant reduction in biomass wastage.

Figs. 5 and 6 show the diurnal variations in both biogas production rates and biogas content, respectively, at SRTs of 3, 5, 10 and 20 days. The simulations were done at an OLR of 6.5 gCOD/L-d, as this OLR is fairly reasonable for mesophilic methanogenic anaerobic digesters [30]. As shown in Figs. 5a and 6a, at SRT of 3 days, the hydrogen production rate and hydrogen content in the biogas increased initially to 10 L/d and 70%, respectively, over a period of 10 days, and stabilized thereafter reaching a minimum of 9 L/d and 67%, respectively. The average methane production rate and methane content in the biogas where 0.5 L/d and 4%, respectively. At an SRT of 5 days (see Figs. 5b and 6b), the hydrogen production rate and hydrogen content in the biogas increased initially to 9 L/d and 65%, respectively, over a period of 15 days (3 turnovers of SRT), and declined thereafter, reaching a minimum of 7 L/d and 55%, respectively. On the other hand, over the same period, methane production rate and methane content decreased to

Table 6 – Summary of process parameters at different SRTs for unsuppressed methanogens.

	SRT = 3 days	SRT = 5 days	SRT = 10 days	SRT = 20 days
Gas Flow Rate (L/d)	15 ± 1	12 ± 1	7 ± 1	7 ± 0.4
H_2 (%)	68 ± 5	62 ± 5	18 ± 16	4 ± 5
CH_4 (%)	4 ± 3	9 ± 4	46 ± 13	55 ± 3
VSS (mg/L)	1700 ± 480	2400 ± 440	4000 ± 350	6200 ± 300
VFAs (mg/L)	1600 ± 90	1600 ± 100	1500 ± 100	1400 ± 100
H_2 (L/d)	10 ± 1	8 ± 1	1 ± 1	0.2 ± 0.3
CH_4 (L/d)	0.5 ± 0.3	1.1 ± 0.3	3 ± 0.6	4 ± 0.4
Alkalinity (mg/L)	700	700	700	700
VFA/alkalinity ratio	2.3	2.3	2.1	2
pH	5.5 ± 0.1	5.5 ± 0.1	5.4 ± 0.2	5.3 ± 0.2

Note: values represent average ± standard deviation.

1 L/d and 7%, respectively, and then they both increased gradually up to 1.5 L/d and 15%, respectively. For SRTs 10 days and 20 days, the adverse impact of SRT on hydrogen production was more significant where both hydrogen production rate and hydrogen content decreased drastically after 2 months of continuous operation. At SRT of 10 days, the hydrogen production rate and hydrogen content dropped down to 0.5 L/d and 3%, respectively, while at SRT of 20 days they decreased down to 0.2 L/d and 1% with a drastic increase in methane content up to 55%. Table 6 summarizes the main process parameters at the 4 different SRTs. It is clearly evident that up to an SRT of 5 days, hydrogen production was predominant with a reasonable deterioration in the production rate by 20%. However, screening of the data of VFAs reported in Table 5 reveals that the VFAs concentration at SRTs of 10 days and 20 days dropped by only 10% compared to their values at SRTs of 3 days and 5 days which shows that the system was still producing hydrogen at the same conversion efficiency but hydrogen was eventually consumed by the hydrogenotrophic methanogens. This hypothesis will be investigated in the following section that discusses the biomass distribution in the system considering the main three bacterial groups associated with the process (hydrogen producers, acetoclastic methanogens and hydrogenotrophic methanogens).

3.3. *Biomass distribution*

As depicted in Fig. 7, hydrogen producers were the predominant bacterial group in the reactor and they constituted approximately 90% of the total biomass concentration. The concentration of hydrogen producers ranged from 1300 mgVSS/L to 15500 mgVSS/L for OLRs ranging from 6.5 gCOD/L-d to 103 gCOD/L-d, while the concentration of both acetoclastic and hydrogenotrophic methanogens did not exceed 20 mgVSS/L and 75 mgVSS/L, respectively over the same range of OLRs. It is noteworthy that the total concentration of methanogens was less than 1% of the reactor's total biomass concentration at the four OLRs. In addition, the diurnal variation in biomass concentration shown in Fig. 7 (a, b, c and d) followed a similar trend to that observed in Fig. 3 (a, b, c, and d) for hydrogen and methane production rates. Moreover, comparing the VFAs values and hydrogen production rates for methanogens-ON and OFF at the four different OLRs confirms that absence of both acetoclastic methanogens (converts VFAs to methane) and hydrogenotrophic methanogens (utilize hydrogen and carbon dioxide to form methane) where, both VFAs and hydrogen production rates were not affected.

Fig. 8 shows the impact of different SRTs on the bacterial community. As shown in Fig. 8 (a, b, c and d), the concentration of acetoclastic methanogens decreased drastically from their initial concentration at startup to less than 20 mgVSS/L over a period of 30 days. According to the SRT_{design} calculations presented earlier, it is evident that up to SRT of 20 days, acetoclastic methanogens are completely washed out of the system which is confirmed by the fairly constant VFAs concentration for SRTs of up to 20 days (refer to Table 6). However, the concentration of hydrogenotrophic methanogens showed a different trend where, at SRT of 5 days and 10 days their concentration increased over a period of 90 days to reach a maximum of 180 mgVSS/L. While at an SRT of 20 days, a rapid increase in hydrogenotrophic methanogens was detected with a maximum concentration of 1000 mgVSS/L accounting 50% of the hydrogen producers' concentration of 2000 mgVSS/L. Extending the SRT beyond 3 days increased the biomass concentration (including the concentration of hydrogen producers) in the hydrogen reactor but had an adverse impact on the process due to the

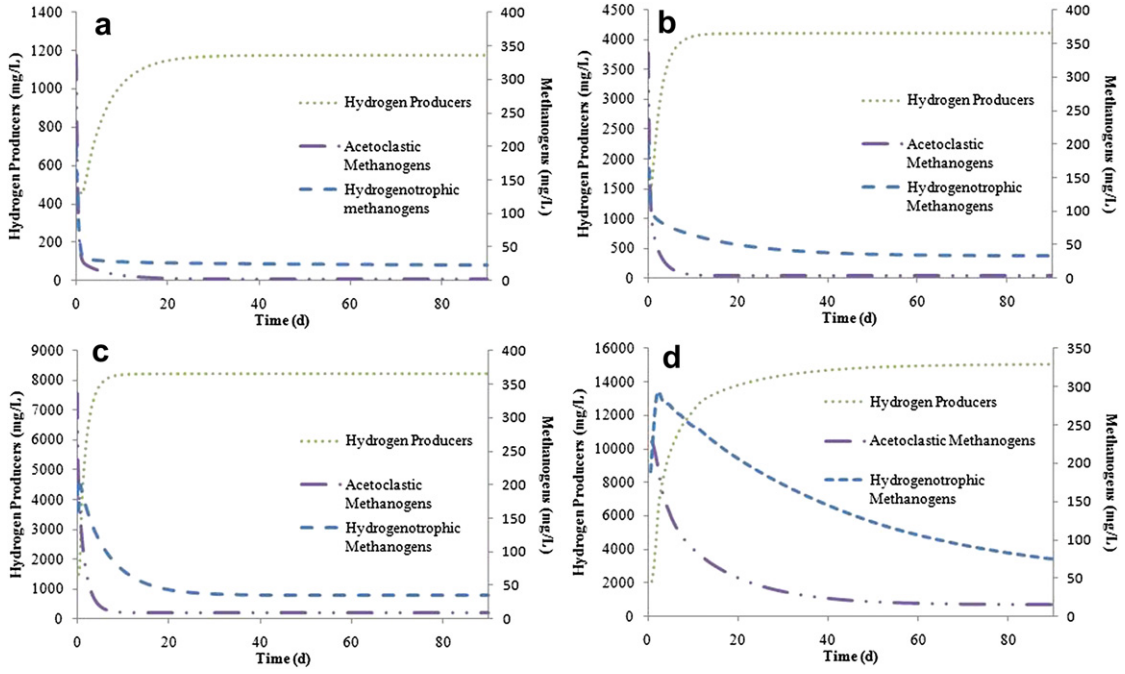

Fig. 7 – Major bacterial communities concentration (mgVSS/L) at: a) OLR-1 = 6.5 gCOD/L-d, b) OLR-2 = 25.7 gCOD/L-d, c) OLR-3 = 51.4 gCOD/L-d, d) OLR-4 = 103 gCOD/L-d.

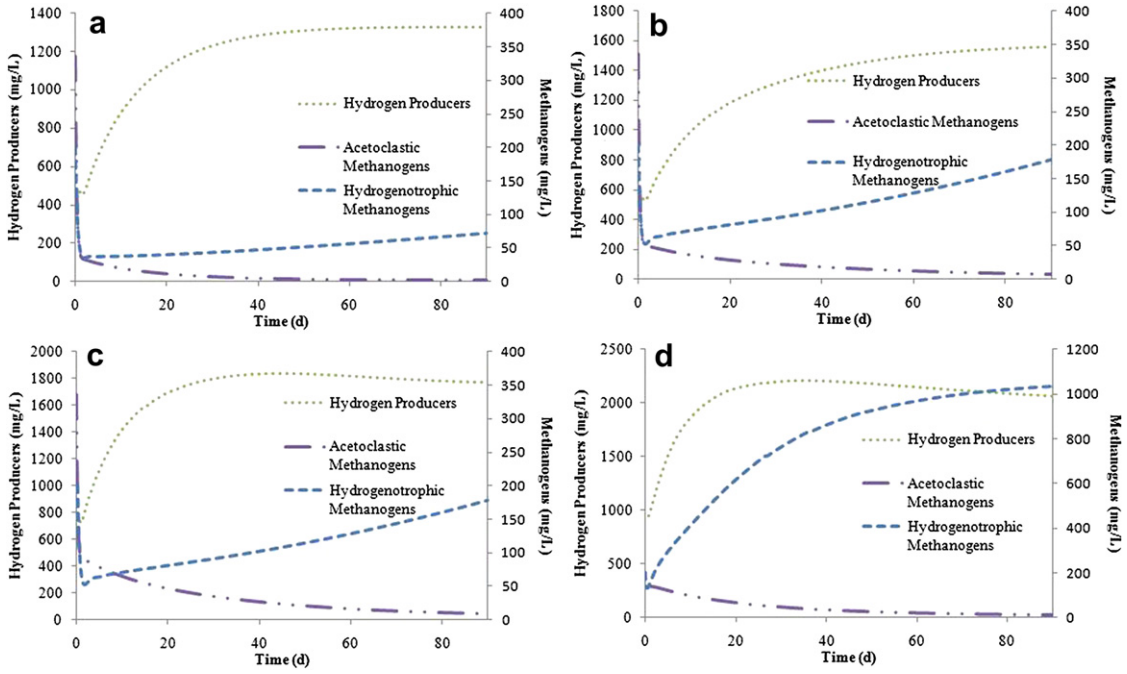

Fig. 8 – Major bacterial communities concentration (mgVSS/L) at: a) SRT = 3 days, b) SRT = 5 days, c) SRT = 10 days, d) SRT = 20 days.

consumption of the hydrogen gas by hydrogen utilizing bacteria (hydrogenotrophic methanogens) that has an SRT_{design} of 4.2 days as calculated earlier.

4. Summary and conclusions

The impact of pre-treatment for methanogens suppression on biohydrogen production in the IBRCS was evaluated in this study. The following conclusion can be drawn:

- Dynamic simulations at four different OLRs ranging from 6.5 to 103 gCOD/L-d and at HRT of 8 h and SRT of 1–2 d have shown that without any pre-treatment, the system was capable of washing out methanogens and enriching hydrogen producers. The average methane gas content in the reactor's headspace was 4% after 7 days of continuous operation, decreasing sharply to less than 0.5%, while the maximum reduction in hydrogen gas was <10%. The hydrogen gas content in the headspace ranged from 65% to 72%.
- Simulating the impact of extended SRT ranging from 3 days to 20 days on the performance of the IBRCS revealed that up to an SRT of 5 days hydrogen production was predominant with a reasonable deterioration in the production rate by 20%.
- Biomass distribution showed that at SRTs up to 20 days, acetoclastic methanogens were naturally eliminated. However, hydrogenotrophic methanogens had a significant impact on the overall hydrogen production rate where most of the hydrogen gas produced was consumed at SRTs of 10 days and 20 days that are considered long enough compared to SRT_{design} of 4.2 days required for hydrogenotrophic methanogens.

Thus, this study opens the door for using mixed or pure cultures for biohydrogen production without any pre-treatment and eliminates one of the major misconceptions that hindered real feedstock utilization due to potential microbial contamination.

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