2012

Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage

Noha Nasr, *Western University*

Elsayed Elbeshbishy, *Western University*

Hisham Hafez

George Nakhla, *Western University*

M. Hesham El Nagggar, *Western University*

Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage

Noha Nasr^a, Elsayed Elbeshbishy^a, Hisham Hafez^{b,*}, George Nakhla^{a,c}, M. Hesham El Naggar^a

^a Department of Civil Engineering, The University of Western Ontario, London, Ontario, Canada N6A 5B9

^b GreenField Ethanol Inc., Chatham, Ontario, Canada N7M 5J4

^c Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario, Canada N6A 5B9

ARTICLE INFO

Article history:

Received 13 December 2011

Received in revised form 3 February 2012

Accepted 6 February 2012

Available online 14 February 2012

Keywords:

Anaerobic digestion

Single-stage

Two-stage comprising hydrogen and methane

Thin stillage

Hydrogen

ABSTRACT

A comparative evaluation of single-stage and two-stage anaerobic digestion processes for biomethane and biohydrogen production using thin stillage was performed to assess the impact of separating the acidogenic and methanogenic stages on anaerobic digestion. Thin stillage, the main by-product from ethanol production, was characterized by high total chemical oxygen demand (TCOD) of 122 g/L and total volatile fatty acids (TVFAs) of 12 g/L. A maximum methane yield of 0.33 L CH₄/gCOD_{added} (STP) was achieved in the two-stage process while a single-stage process achieved a maximum yield of only 0.26 L CH₄/gCOD_{added} (STP). The separation of acidification stage increased the TVFAs to TCOD ratio from 10% in the raw thin stillage to 54% due to the conversion of carbohydrates into hydrogen and VFAs. Comparison of the two processes based on energy outcome revealed that an increase of 18.5% in the total energy yield was achieved using two-stage anaerobic digestion.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Some processes employed in the production of renewable biofuels, such as, bioethanol can result in significant pollution problems. In a typical bioethanol plant process, up to 20 L of stillage can be generated during fermentation for each liter of ethanol produced (van Haandel and Catunda, 1994). Thin stillage is characterized by high total chemical oxygen demand (TCOD) of up to 122 g/L, biological oxygen demand (BOD) of up to 70 g/L, volatile solids (VS) of 60 g/L (Schaefer and Sung, 2008; Nasr et al., 2011) and total carbohydrates of 65% (based on dry mass) (Mustafa et al., 2000). Therefore, it is a strong candidate for anaerobic digestion. Usually, due to solids build up and toxicity to yeast by lactic acid, acetic acid, glycerol and sodium, less than 50% of thin stillage is recycled as fermentation broth (called backset in the corn-to-ethanol industry) (Egg et al., 1985; Shojaosadati et al., 1996; Julian et al., 1990; Pejin et al., 2009).

Abbreviations: TCOD, total chemical oxygen demand; BOD, biological oxygen demand; VS, volatile solids; STP, standard temperature and pressure ($T = 0^{\circ}\text{C}$ and $P = 1\text{ atm}$); UASB, upflow anaerobic sludge blanket; TVFAs, total volatile fatty acids; ADS, anaerobic digester sludge; TSS, total suspended solids; VSS, volatile suspended solids; AADS, acclimatized anaerobic digester sludge; HRT, hydraulic retention time; SRT, solid retention time; S^0/X^0 , substrate-to-biomass ratio; TCD, thermal conductivity detector.

* Corresponding author. Tel.: +1 519 784 6230; fax: +1 519 352 9559.

E-mail address: h.hafez@greenfieldethanol.com (H. Hafez).

In a single-stage anaerobic digestion, Stover et al. (1984) observed promising performances from mesophilic digestion of thin corn stillage (64.5 g TCOD/L; 32.2 gTS/L) in both suspended growth and fixed-film systems with a methane yield ranging from 0.22 to 0.33 m³/kg TCOD_{removed} (STP) that could replace 60% of the daily energy requirement of the bioethanol plant. One pilot scale upflow anaerobic sludge blanket (UASB) reactor achieved 76% TCOD removal with 0.33 m³ CH₄/kgTCOD removed. It was also used for a corn ethanol plant as a stillage pretreatment step before aerobic trickling filters; however influent wastewater TCOD was only 3.6 g/L (Lanting and Gross, 1985).

Separating the acidogenic and methanogenic steps in the anaerobic digestion process, provides enhanced stability to the different groups of microorganisms and better process control (Demirel and Yenigun, 2002). The purpose of a two-stage anaerobic digestion system is not only to further degrade waste, but also to extract more net energy from the system (Thompson, 2008). In a single-stage anaerobic digestion process, a variety of higher organic acids, such as propionic, butyric, and lactic, as well as alcohols and ketones, are also formed during the breakdown of the organic substrates by acidogens. However, in a well operated process, these products are mostly converted to acetic acid and hydrogen, which, in turn, are converted to methane gas (Cooney et al., 2007). On the other hand, in a two-stage anaerobic digestion process, the end products from acidification stage using thin stillage are ideal for anaerobic treatment with total volatile fatty acids (TVFAs) that can reach 29.5 gCOD/L (Pavan et al., 2000; Nasr et al., 2011).

Vinas et al. (1993) achieved a methane production yield of 0.31 L/gCOD_{removed} (STP) in a two-stage process with an increase of 13% over the single-stage process using a cellulosic material as the substrate. Also, Rincon et al. (2009) achieved an increase of 10% using olive mill solid residue as the substrate. Although both studies used acidification stage as a pretreatment for anaerobic digestion, they did not consider biohydrogen production.

Despite of their higher loading rates, improved process stability and flexibility, there are relatively few commercial two-stage anaerobic digestion units. The added complexity and expense of building and operating commercial two-stage systems have so far counteracted the yield and rate enhancements (Rapport et al., 2008). The theoretical higher biogas yields have also been questioned since the acidogenic phase separation prevents the hydrogen to methane pathway (Reith et al., 2003).

The main objective of this research is to compare and evaluate the methane production from thin stillage in single-stage and two-stage anaerobic digestion processes, by investigating the effect of the acidogenic stage with hydrogen production on the methane production in batch studies under mesophilic conditions, and to determine if there is a significant difference in potential energy yields between single-stage and two-stage anaerobic digestion systems.

2. Methods

2.1. Seed sludge

Anaerobic digester sludge (ADS) was collected from the primary methane digester at Guelph's wastewater treatment plant (Guelph, Ontario, Canada) and was used as seed sludge for the single-stage anaerobic digestion and the second stage of the two-stage anaerobic digestion for methane production. The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 22.9 and 13.2 g/L, respectively. Acclimatized anaerobic digester sludge (AADS) was collected from a continuous-flow biohydrogen system (Nasr et al., 2011). The 15 L/d continuous-flow system was run for 10 days, using 30 g/L glucose as a substrate and heat pretreated ADS as a seed at a hydraulic retention time (HRT) of 8 h and solids retention time (SRT) of 42 h. The TSS and VSS concentrations of the AADS were 10.9 and 9.4 g/L, respectively.

2.2. Feed (substrate)

Raw thin stillage was used as the substrate to assess its hydrogen and methane production potentials. For the single-stage methane production and the first stage hydrogen production, raw thin stillage was used as the substrate with TCOD, TVFAs, TSS, and VSS of 122, 12.3, 36.9, and 35.3 g/L, respectively. Detailed characteristics of the raw thin stillage have been reported elsewhere (Nasr et al., 2011). Hydrogen batch tests were tested at an initial substrate-to-biomass ratio (S^0/X^0) of 4, 6, and 8 gCOD/gVSS based on the TCOD of the thin stillage and seed sludge VSS concentration

(Nasr et al., 2011). After the hydrogen production stage, the bottles of the three different S^0/X^0 ratios were left for three hours to settle and the supernatant was then used as substrate for the second stage methane production. TCOD of the supernatants from S^0/X^0 ratios of 4, 6, and 8 gCOD/gVSS described below were 49.6, 51.5, and 53.3 g/L, respectively.

2.3. Batch experiments

Hydrogen and methane batch anaerobic experiments were conducted in serum bottles with a liquid volume of 250 mL and head space volume of 60 mL. Table 1 shows the volumes of substrates and sludges used in bottles and initial pH for each stage. For hydrogen production as a first stage, the experiments were conducted in triplicates for initial (S^0/X^0) ratios of 4, 6 and 8 gTCOD_{substrate}/gVSS_{seed} using AADS as the seed and raw thin stillage as the substrate (Nasr et al., 2011). For methane production, the experiments were conducted in triplicates for an initial S^0/X^0 ratio of 2 gCOD/gVSS using ADS as the seed and the supernatant from the hydrogen production stage as the substrate. The volumes of thin stillage and supernatant as substrates and ADS and AADS as seeds used in batches were calculated using the following equation:

$$S/X = [V_{\text{substrate}}(L) * \text{TCOD}_{\text{substrate}}(\text{g/L})] / [V_{\text{sludge}}(L) * \text{VSS}_{\text{sludge}}(\text{g/L})] \quad (1)$$

where $V_{\text{substrate}}$ is the volume of substrate and V_{sludge} is the volume of sludge. Buffer (NaHCO_3) with concentrations of 5 g/L and 12 g/L were added for pH control in both hydrogen and methane batches, respectively. The initial pH for the mixed solution in each bottle was subsequently adjusted using HCl or NaOH and measured to be 5.47 ± 0.04 for hydrogen batches and 7.17 ± 0.07 for methane batches.

Initially, 10 mL samples of the mixtures were collected. The head space was flushed with oxygen-free nitrogen gas for a period of 2 min and capped tightly with rubber stoppers. The bottles were then placed in a swirling-action shaker (Max Q4000, Incubated and Refrigerated Shaker, Thermo Scientific, CA) operating at 180 rpm and maintained at a temperature of 37 °C. Two control bottles of seed material only, without substrate, were prepared using ADS for methane production runs and one control bottle using AADS for hydrogen production run. Final samples were taken at the end of the batch experiment.

2.4. Analytical methods

The biogas production was measured by releasing the gas pressure in the vials using appropriately sized glass syringes (Perfektum; Popper & Sons Inc., NY, USA) in the 5–100 mL range to equilibrate with the ambient pressure (Owen et al., 1979). The composition of biogas including hydrogen, methane, and nitrogen was determined by employing a gas chromatograph (Model 310, SRI Instruments, Torrance, CA) equipped with a thermal

Table 1
Volumes of substrate and sludge used in batches.

			S^0/X^0 (gCOD/gVSS)	$V_{\text{substrate}}$ (mL)	V_{sludge} (mL)	pH _{initial}
Two-stage CH ₄ production	Single-stage CH ₄ production (using ADS)		2	45	205	7.17 ± 0.05
	1st stage H ₂ production (Run A)	A1	4	54	196	5.47 ± 0.05
		A2	6	73	177	5.48 ± 0.02
	(using AADS)	A3	8	89	161	5.50 ± 0.01
	2nd stage CH ₄ production (Run B)	B1	2 from	4	67	7.18 ± 0.06
		B2		6	65	7.16 ± 0.08
	(using ADS)	B3		8	64	7.18 ± 0.05

conductivity detector (TCD) and a molecular sieve column (Mole sieve 5A, mesh 80/100, 6 ft × 1/8 in). The temperatures of the column and the TCD detector were 90 and 105 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL/min. TVFAs, TCOD, and SCOD were measured using HACH methods. TSS and VSS concentrations were analyzed using standard methods (APHA, 1995). Soluble parameters were determined after filtering the samples through 0.45 µm filter paper.

2.5. Data analysis

Hydrogen and methane gas productions were calculated from head space measurements of gas composition and the total volume of biogas produced at each time interval, using the mass balance equation:

$$V_{X,i} = V_{X,i-1} + C_{X,i} * V_{G,i} \quad (2)$$

Where $V_{X,i}$ and $V_{X,i-1}$ are cumulative hydrogen or methane gas volumes at the current (i) and previous (i – 1) time intervals, $V_{G,i}$ is the total biogas volume in the current and previous time intervals, $C_{X,i}$ is the fraction of hydrogen or methane gas in the head-space of the bottle measured using gas chromatography in the current time interval.

3. Results and discussion

3.1. Biogas production

The first stage (i.e. acidogenic stage) was carried out with three different S^0/X^0 ratios of 4, 6, and 8 gCOD/gVSS (runs: A1, A2, and A3) as described in detail by Nasr et al. (2011). Fig. 1 shows the hydrogen production rates achieved for runs A1, A2, and A3 with ultimate hydrogen production potentials of 1676, 1974, and 550 mL, respectively. It can be inferred from the Figure that as the S^0/X^0 ratio increased from 4 to 6 gCOD/gVSS, hydrogen production rate increased from 47 mL/h to 62 mL/h, respectively, after which it decreased significantly to 28 mL/h at S^0/X^0 ratio of 8 gCOD/gVSS. This trend is consistent with another study that observed the same pattern of maximum hydrogen production at food to microorganism (F/M) ratio of 6 gCOD/gVSS-d followed by a sharp decline at higher F/M ratios (Hafez et al., 2010a).

It is noteworthy that in the single-stage anaerobic digestion process, there was no hydrogen gas detected with methane gas production. The COD degradation was 80% complete in the single-stage experiments (A runs) after 28 days while in the two-stage experiments (B runs), it took only 17.5, 17.8, and 16.7 days to reach 80% degradation for the three runs B1, B2, and B3, respectively. Therefore, a shorter SRT can be attained in the two-stage anaerobic

Fig. 1. H_2 production rates for the acidogenic step in the two-stage batches.

Table 2

Samples characteristics for the single-stage anaerobic digestion batches at S^0/X^0 ratio of 2 gCOD/gVSS.

CH ₄ mL	pH _{final}	TCOD _i mg/L	CH ₄ yield	
			$L_{CH_4}/gCOD_{substrate-initial}$	$L_{CH_4}/L_{thin\ stillage}$
1299	5.05 ± 0.15	35483	0.3	29

digestion process leading to improvement in the overall performance of the anaerobic digestion. The final pHs for the mixed solution in each bottle were measured and found to be 7.56 ± 0.01 for methane runs and 5.05 ± 0.15 for the hydrogen runs.

3.2. Hydrogen and methane yields

Tables 2 and 3 show the summary for initial and final batches data in both single-stage and two-stage anaerobic digestion experiments. Fig. 2 shows the methane yield during the single-stage and two-stage anaerobic digestion of thin stillage. Standard deviation values were less than 10% for all experimental data. In the two-stage anaerobic digestion, the methane yields based on COD removed were 321, 333, and 317 mL $CH_4/gCOD_{removed}$ (STP) for the methanogenic batches of runs B1, B2, and B3, respectively. On the other hand, a methane yield of only 268 mL $CH_4/gCOD_{removed}$ (STP) was achieved in the single-stage experiment. The maximum methane yield of 333 mL/gCOD_{removed} (STP) was 24% higher than the yield achieved in the single-stage experiment compared to an increase of 9.8% achieved by Rincon et al. (2009) and 13.3% by Vinas et al. (1993).

Fig. 3 shows the maximum methane production rates for the single and two-stage anaerobic digestion processes. The methane production rate in the two-stage anaerobic digestion was higher than that in the single-stage process. Maximum methane production rates of 3.67, 3.88, and 3.78 mL CH_4/h were achieved in the

Table 3

Samples characteristics for the two-stage anaerobic digestion batches (methanogenic step) at S^0/X^0 ratio of 2 gCOD/gVSS.

From S^0/X^{0a}	pH _{final}	CH ₄ mL	TCOD _i mg/L	CH ₄ yield $L_{CH_4}/gCOD_{substrate-initial}$
4	7.57 ± 0.01	1020	27060	0.37
6	7.55 ± 0.01	1073	27780	0.38
8	7.54 ± 0.02	1035	26853	0.36

^a From the acidogenic stage (hydrogen production).

Fig. 2. CH_4 yield for single and two-stage batches.

Fig. 3. CH₄ production rates for single and two-stage batches.

three runs B1, B2, and B3, respectively, which were 38% higher than the 2.82 mL CH₄/h in the single-stage experiment.

In the single-stage anaerobic digestion, the methane yield based on the thin stillage COD added was 0.26 L/gCOD_{added} (STP) as compared to 0.33 L/gCOD_{added} (STP) in the two-stage anaerobic digestion process. Lee et al. (2011) reported a methane yield of 0.22 L/gCOD_{added} (STP) using corn thin stillage of TCOD 131 g/L in a single-stage anaerobic digestion process. After correcting for the methane produced from the blank (inoculum only), the volumetric yield of thin stillage used was 26 L CH₄/L_{thin stillage} (STP) in the single-stage experiment. Yields based on thin stillage used were not calculated for the two-stage anaerobic digestion since the substrate used was the supernatant from the acidogenic step and not raw thin stillage.

In the first step of the two-stage anaerobic digestion process, hydrogen yields of 557, 478, and 247 mL/gCOD_{removed} were achieved in the acidogenic step for runs A1, A2, and A3, respectively (Nasr et al., 2011).

3.3. Volatile fatty acids

After the hydrolysis stage, the acid forming bacteria ferment glucose to produce a mixture of VFAs of acetic, butyric, and propionic acids (Batstone et al., 2002) according to the reactions:

The TVFAs measured for the final samples after the acidogenic step were 24, 29.5, and 21.4 gCOD/L for S°/X° ratios of 4, 6, and 8 gCOD/gVSS, respectively. The hydrogen potential from S°/X° ratio of 8 gCOD/gVSS (run A3) was around one third for the other two S°/X° ratios (runs A1 and A2), and the hydrogen yield based on COD removed was less than half the hydrogen yields in runs A1 and A2. However, the final TVFAs for run A3 were 87.5% and 72.4% of the final TVFAs for runs A1 and A2, respectively.

It is noteworthy that in the methanogenic phase of the two-stage anaerobic digestion process, the concentration of TVFAs in the influent accounted for 53.6% of the TCOD, while TVFAs in the single-stage anaerobic digestion influent was only 10% of the TCOD. Since it is widely known that in methanogenesis 67% of the methane is produced by acetate-utilizing methanogens and 33% is by hydrogenophilic methanogens (Kotsyurbenko et al.,

2004), the importance of separating the acidification phase in a two-stage anaerobic digestion process, is emphasized.

3.4. Anaerobic biodegradability

The extent of anaerobic biodegradability (BD_{CH₄}) of thin stillage can be calculated from the experimental methane yield, taking into consideration the theoretical methane yield of 0.35 L/gCOD (STP) (Raposo et al., 2011), i.e.:

$$BD_{CH_4}(\%) = (B_{0\text{exp}}/B_{0\text{th}}) * 100 \quad (7)$$

where B_{0exp} is the experimental methane potential (L) and B_{0th} is the theoretical methane potential (L) based on the initial TCOD of thin stillage. The anaerobic biodegradability of thin stillage was 88.2% in the single-stage anaerobic digestion and 99% in case of the two-stage anaerobic digestion. This emphasizes that indeed the acidogenic step enhanced the anaerobic biodegradability of thin stillage.

Anaerobic digestion is commonly described as a first-order reaction, and can be expressed as:

$$\ln[(B_0 - B)/B_0] = -k \cdot t \quad (8)$$

where *t* is the digestion time (d), *k* is the first order kinetic constant (d⁻¹), B₀ is the methane potential at the end of the experiment, and *B* is the methane production at time *t* (Chen and Hashimoto, 1978). In the single-stage anaerobic digestion, the value of the kinetic constant *k* was 0.05 d⁻¹, while in the two-stage anaerobic digestion a kinetic constant of 0.07 d⁻¹ was achieved.

3.5. Hydrogen and methane energy yields

COD destruction efficiencies during the methanogenic stage were relatively low at 43–53% due to the high initial S°/X° value of 2 gCOD/gVSS, and accordingly are not representative of continuous flow digestion which operates at SRTs of 15 days and loadings of 0.15–0.30 gCOD/gVSS-d. To compare the performance of single-stage vs. two-stage digestion, energy outcome from both systems was calculated using the following assumptions: theoretical methane yield of 0.35 L CH₄/gCOD_{consumed} (STP), energy content of hydrogen and methane of 142 kJ/g_{hydrogen} (equivalent to 12.8 kJ/L_{hydrogen}) (Cai et al., 2004) and 50 kJ/g_{methane} (equivalent to 35.8 kJ/L_{methane}) (Ogden, 2002), respectively, and COD destruction efficiency of 80% in the single stage anaerobic digestion process (Elbeshbishy and Nakhla, 2011) and an overall COD destruction efficiency of 90% in the two-stage process (Blonskaja et al., 2003; Vinas et al., 1993; Hafez et al., 2010b). One liter of thin stillage in a single-stage continuous-flow anaerobic digestion process generates 38.5 L of methane which is equivalent to 1380 kJ. On the other hand, one liter of thin stillage in a two-stage continuous-flow anaerobic digestion process generates 19.5 L of hydrogen in the first stage and 38.7 L of methane in the second stage which is equivalent to a total of 1635 kJ with an 18.5% increase in the energy yield. Similarly, Luo et al. (2011) observed an 11% increase in overall energy yield in a thermophilic two-stage hydrogenic and methanogenic digestion of thin stillage as compared to a single-stage thermophilic system.

The advantages of two-stage over single-stage mesophilic digestion of waste activated sludge (i.e. higher organic stabilization and gasification rates and efficiencies, enhanced net energy production, and greater pathogen kills) have been known for decades (Ghosh et al., 1995). The fundamental difference between the conventional two-stage anaerobic digestion process with acidification as a first stage and a two-stage process with hydrogen production in the first stage is the optimization of hydrogen production with respect to environmental and operational conditions in the latter one. Many studies investigated the pH and HRT effect on hydrogen

production and concluded that the optimal pH is 5.5 and optimal HRT is in the range of 3–8 h (Li and Fang, 2007). Recently, Kvesitadze et al. (2012) has confirmed that thermophilic hydrogen production from the organic fraction of municipal solid wastes in batches at pH 5.5 peaked at 8 h. On the other hand, A wide range of HRT (2–5 days) was reported for the acidification stage with negligible hydrogen production (Elbeshbishy and Nakhla, 2011; Takashima and Tanaka, 2010).

4. Conclusions

The use of two-stage digestion for the treatment of thin stillage led to an increase in the TVFAs to TCOD ratio from 10% to 56.8% due to the acidification process during hydrogen production in the first stage. The methane yield in the anaerobic digestion stage increased from 0.26 L CH₄/g COD_{added} to 0.33 L CH₄/g COD_{added}. Comparison of energy outcome from both digestion scenarios revealed that an overall increase of 18.5% in energy yield can be achieved in the two-stage digestion due to the enhancement in methane yield and the additional energy produced from hydrogen gas.

References

- APHA, AWWA, WEF, 1995. Standard methods for examination of water and wastewater. 19th ed.
- Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., et al., 2002. Anaerobic digestion model No.1, IWA task group for mathematical modelling of anaerobic digestion processes. *Water Sci. Technol.* 45 (10), 65–73.
- Blonskaja, V., Menert, A., Vilu, R., 2003. Use of two-stage anaerobic treatment for distillery waste. *Adv. Environ. Res.* 7 (3), 671–678.
- Cai, M.L., Liu, J.X., Wei, Y.S., 2004. Enhanced biohydrogen production from sewage sludge with alkaline pretreatment. *Environ. Sci. Technol.* 38 (11), 3195–3202.
- Chen, Y.R., Hashimoto, A.G., 1978. Kinetics of methane fermentation. *Biotechnol. Bioeng. Symp.* 8, 269–283.
- Cooney, M., Maynard, N., Cannizzaro, C., Benemann, J., 2007. Two-phase anaerobic digestion for production of hydrogen-methane mixtures. *Bioresour. Technol.* 98 (14), 2641–2651.
- Demirel, B., Yenigun, O., 2002. Two-phase anaerobic digestion processes: a review. *J. Chem. Technol. Biotechnol.* 77 (7), 743–755.
- Egg, R.P., Sweeten, J.M., Coble, C.G., 1985. Grain sorghum stillage recycling: effect on ethanol yield and stillage quality. *Biotechnol. Bioeng.* 27, 1735–1738.
- Elbeshbishy, E., Nakhla, G., 2011. Comparative study of the effect of ultrasonication on the anaerobic biodegradability of food waste in single and two-stage systems. *Bioresour. Technol.* 102 (11), 6449–6457.
- Ghosh, S., Buoy, K., Dressel, L., Miller, T., Wilcox, G., Loos, D., 1995. Pilot- and full-scale two-phase anaerobic digestion of municipal sludge. *Water Environ. Res.* 67, 206–214.
- Hafez, H., Nakhla, G., El Naggar, M.H., Elbeshbishy, E., Baghchehsaraee, B., 2010a. Effect of organic loading on a novel hydrogen bioreactor. *Int. J. Hydrogen Energy* 35 (1), 81–92.
- Hafez, H., Nakhla, G., El Naggar, H., 2010b. An integrated system for hydrogen and methane production during landfill leachate treatment. *Int. J. Hydrogen Energy* 35 (10), 5010–5014.
- Julian, G.S., Bothast, R.J., Krull, L.H., 1990. Glycerol accumulation while recycling thin stillage in corn fermentations to ethanol. *J. Ind. Microbiol.* 5 (6), 391–394.
- Kotsyurbenko, O.R., Chin, K.J., Glagolev, M.V., Stubner, S., Simankova, M.V., Nozhevnikova, A.N., et al., 2004. Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog. *Environ. Microbiol.* 6 (11), 1159–1173.
- Kvesitadze, G., Sadunishvili, T., Dudaui, T., Zakariashvili, N., Partskhaladze, G., Ugrehelidze, V., et al., 2012. Two-stage anaerobic process for bio-hydrogen and bio-methane combined production from biodegradable solid wastes. *Energy* 37, 94–102.
- Lanting, J., Gross, R.L., 1985. Anaerobic pretreatment of corn ethanol production wastewater. In: *Proceedings of the 40th Industrial Waste Conference*, Purdue University, West Lafayette, Indiana, Butterworth, Boston, pp. 905–914.
- Lee, P.H., Bae, J., Kim, J., Chen, W.H., 2011. Mesophilic anaerobic digestion of corn thin stillage: a technical and energetic assessment of the corn-to-ethanol industry integrated with anaerobic digestion. *J. Chem. Technol. Biotechnol.* 86 (12), 1514–1520.
- Li, C., Fang, H.H.P., 2007. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. *Environ. Sci. Technol.* 37 (1), 1–39.
- Luo, G., Xie, L., Zhou, Q., Angelidaki, I., 2011. Enhancement of bioenergy production from organic wastes by two-stage anaerobic hydrogen and methane production process. *Bioresour. Technol.* 102 (18), 8700–8706.
- Mustafa, A.F., McKinnon, J.J., Christensen, D.A., 2000. The nutritive value of thin stillage and wet distillers' grains for ruminants: a review. Department of animal and poultry science, University of Saskatchewan, Saskatchewan, Canada.
- Nasr, N., Elbeshbishy, E., Hafez, H., Nakhla, G., El Naggar, M.H., 2011. Bio-hydrogen production from thin stillage using conventional and acclimatized anaerobic digester sludge. *Int. J. Hydrogen Energy* 36 (20), 12761–12769.
- Ogden, J., 2002. Hydrogen – The fuel of the future? *Phys. Today* 55, 69–75.
- Owen, W.F., Stuckey, D.C., Healy, J.B., Young, L.Y., McCarty, P.L., 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.* 13, 485–492.
- Pavan, P., Battistoni, P., Cecchi, F., Mata-Alvarez, J., 2000. Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid wastes): performance and kinetic study. *Water Sci. Technol.* 41 (3), 111–118.
- Pejin, D., Mojovic, L., Grujic, O., Pejin, J., Rakin, M., 2009. The bioethanol production with the thin stillage recirculation. *Chem. Industry Chem. Eng. Q.* 15 (1), 49–52.
- Raposo, F., Fernandez-Cegri, V., De la Rubia, M.A., Borja, R., Beline, F., Cavinato, C., et al., 2011. Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J. Chem. Technol. Biotechnol.* 86, 1088–1098.
- Rapport, J., Zhang, R., Jenkins, B.M., Williams, R.B., 2008. Current anaerobic digestion technologies used for treatment of municipal organic solid waste. Contractor's report California Environmental Protection Agency, California.
- Reith, J.H., Wijffels, R.H., Barten, H., 2003. Bio-Methane and Bio-Hydrogen: Status and Perspectives of Biological Methane and Hydrogen Production. Dutch Biological Hydrogen Foundation, Netherlands.
- Rincon, B., Borja, R., Martin, M.A., Martin, A., 2009. Evaluation of the methanogenic step of a two-stage anaerobic digestion process of acidified olive mill solid residue from a previous hydrolytic-acidogenic step. *Waste Manag.* 29 (5), 2566–2573.
- Schaefer, S.H., Sung, S., 2008. Retooling the ethanol industry: thermophilic anaerobic digestion of thin stillage for methane production and pollution prevention. *Water Environ. Res.* 80 (2), 101–108.
- Shojaosadati, S.A., Sanaei, H.R., Fatemi, S.M., 1996. The use of biomass and stillage recycle in conventional ethanol fermentation. *J. Chem. Technol. Biotechnol.* 67 (4), 362–366.
- Stover, E.L., Gomathinayagam, G., Gonzalez, R., 1984. Use of methane from anaerobic treatment of stillage for fuel alcohol production. In: *Proceedings of the 39th. Industrial waste conference*, Purdue University, West Lafayette, Indiana, Butterworth, Boston, pp. 95–104.
- Takashima, M., Tanaka, Y., 2010. Application of acidic thermal treatment for one- and two-stage anaerobic digestion of sewage sludge. *Water Sci. Technol.* 62 (11), 2647–2654.
- Thompson, R.S., 2008. Hydrogen production by anaerobic fermentation using agricultural and food processing wastes utilizing a two-stage digestion system. M.E.Sc. Thesis. Department of Biological and Irrigation Engineering, UTAH State University, Logan, Utah.
- van Haandel, A.C., Catunda, P.F.C., 1994. Profitability increase of alcohol distilleries by the rational use of byproducts. *Water Sci. Technol.* 29 (8), 117–124.
- Vinas, M., Martinez, J., Baselli, B., 1993. Advantages of an anaerobic reactor for TMP wastewater with separated acidogenic and methanogenic stages. *Environ. Technol.* 14 (10), 995–1000.