Environmental Engineering and Management Journal

September/October 2008, Vol.7, No.5, xxx-xxx
http://omicron.ch.tuiasi.ro/EEMJ/

"Gh. Asachi" Technical University of Iasi, Romania

EFFECT OF METHANOGENIC INHIBITORS, INOCULA TYPE, AND TEMPERATURE ON BIOHYDROGEN PRODUCTION FROM FOOD COMPONENTS

Anthony S. Danko, Francisco Pinheiro, Ângela A. Abreu, M. Madalena Alves*

Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, 4710-57 Braga, Portugal

Abstract

Dark fermentation hydrogen production from a mixture of food components using two different methods of methanogenic inhibition (autoclaving and BES) and three different temperatures (37, 60, and 70 ºC) was examined in batch assays for two different mixed anaerobic cultures - one suspended sludge (S) obtained from an anaerobic digester and one granular sludge (G) obtained from a brewery wastewater treatment plant. In general, BES-inhibition of sludge was more robust when compared against heat-treated inoculum. Also, hydrogen, VFA, and sCOD production were affected by increases in temperature although the effects were less severe for G than for S. In addition, differences in individual VFAs were observed between the two inocula. S produced more acetate as a percentage of VFA_{TOTAL} compared to G. Conversely, G produced more butyrate compared to S. Differences in the microbial communities were likely responsible for the diverse behaviour of the two inocula.

Keywords: biohydrogen, fermentation, food waste, mixed cultures

1. Introduction

The rise in global pollution and diminishing reserves of fossil fuels has lead to an increase in investment and research into alternative fuel technologies. Hydrogen may be an ideal candidate as an alternative fuel because it is CO_2-neutral and it has the highest energy per mass content of fuels (Boyles, 1984; Kotay and Das, 2007). A wide range of biological technologies can be used to produce hydrogen including photolysis and fermentation. However, rates of hydrogen production from photolysis are less than those from fermentation (Das and Veziroglu, 2001; Levin et al., 2004).

Biohydrogen production from municipal solid waste has been well studied (Lay et al., 1999; Liu et al., 2006; Ueno et al., 2007; Valdez-Vazquez et al., 2005; Valdez-Vazquez et al., 2006). Even though municipal solid waste is comprised of 20-65% kitchen waste (Tchobanoglous et al., 1993), there have only been a few studies concerning hydrogen production from food waste. Okamoto et al. (2000) observed hydrogen production when individual food components such as carrots, cabbage, and rice were used as the substrates (Okamoto et al., 2000). The inoculum used for these experiments was heat treated anaerobic digester sludge. Shin et al., (2004) observed hydrogen production batch reactors using a mesophilic and thermophilic inocula from laboratory scale acidogenic reactor incubated at 37 or 55 ºC (Shin et al., 2004). Hydrogen production has also been observed from semicontinuous reactors using inocula from anaerobic digesters (Shin and Youn, 2005; Kim et al., 2008) or a pilot scale acidogenic reactor (Li et al., 2008). Kim et al. (2008) also used heat treatment to supress methanogenic activity.

Previous studies carried out with other substrates have shown that the different methods to inhibit methanogens can affect hydrogen production (Cheong and Hansen, 2006; Kraemer and Bagley, 2007; Oh et al., 2003; Valdez-Vazquez et al., 2006; Zhu and Béland, 2006). In addition, different inocula

* Author to whom all correspondence should be addressed: Tel.: +351 253604417; fax: +351 253678986. *e-mail :* madalena.alves@deb.uminho.pt

sources and temperature can affect the amount of hydrogen produced (Danko et al., 2008; Lay et al., 1999; Li and Fang, 2007; Lin et al., 2006; Shin et al., 2004; Valdez-Vazquez et al., 2006; Van Ginkel et al., 2001; Yu et al., 2002).

There are conflicting reports in the literature as to effects of temperature on hydrogen production. Several studies have shown that hydrogen yields and rates increase as the temperature increases (Lin and Chang 2004; Morimoto et al., 2004; Valdez-Vazquez et al., 2005; Yu et al., 2002). However, increasing temperature can also have detrimental effects on hydrogen production and rates (Lin et al. 2008; Valdez-Vazquez et al. 2006). Lin et al (2008) showed that even increases of just 5 ºC can impact hydrogen production and rates by as much as 25% (Lin et al., 2008). The work presented herein examines the effect of different methanogenic inhibitors and temperature on hydrogen production for two different anaerobic mixed cultures.

2. Experimental

2.1. Inoculum

A granular sludge and suspended sludge were used in this study. The granular sludge (G) was obtained from an upflow anaerobic sludge blanket reactor treating brewery wastewater located in Oporto, Portugal. The suspended sludge (S) was obtained from a wastewater treatment anaerobic digester supplemented with fat near Coimbra, Portugal.

Prior to use, G was first filtered using a 0.2 mm sieve. Sludge retained on top of the sieve was used as the G inoculum for batch reactors. S was prepared by centrifuging (5,000 rpm), washing in media, and centrifuging (5,000 rpm). Two different methods were used to inhibit methanogenic activity in both G and S: heat treatment by autoclaving (30 min) and bromoethanesulfonate (BES) (25 mM).

2.2. Batch experiments

Batch experiments were performed in 125 mL serum bottles with 20 mL liquid volume containing media, food components, and inoculum. The media contained a bicarbonate buffer with micro- and macro-nutirents as previously described (Abreu et al., 2007; Zehnder et al., 1980). The initial pH of the batch experiments was adjusted to 6.5 by flushing the headspace of each batch reactor with 100% CO_2 for several minutes. The initial amount of biomass used in batch experiments was approximately 10 g/L VS.

The substrate used for these experiments was simulated food waste. The composition of the food waste was prepared by mixing pork lard, cabbage, chicken breast, and potato flakes to simulate lipids, cellulose, protein, and carbohydrates, respectively. Previous research has shown that this simulated food waste adequately represents a real restaurant waste (Neves et al., 2008). The composition of the food waste was manipulated in order to achieve an equal amount of COD for each component of the food waste. This corresponded to 4 g COD/L of each component or a total of 16 g COD/L for the four components in each batch reactor.

The characteristics of the food components are as previously described (Neves et al., 2008). The amount of food components used in the batch experiments are presented in Table 1.

Table 1. Range of the mass (in grams) used for each food component used in the batch experiments

Food Component	***Range (g)****
Fat (Lipids)	0.04 – 0.08
Chicken (Protein)	0.24 – 0-28
Cabbage (Carbohydrates)	1.35 – 1.60
Potato (Cellulose)	0.06 – 0.09

* mass of raw waste expressed in grams of wet mass

Batch cultures were incubated at three different temperatures: 37 ºC (± 2 ºC), 60 ºC (± 2 ºC), and 70 ºC (± 2 ºC). Experiments at each temperature were performed in triplicate.

2.3. Analytical methods

The biogas content of the batch reactors were monitored for hydrogen and methane production using a Hayesep Q column (80/100 mesh) and a Porapak Q (180 to 100 mesh), respectively, with thermal conductivity detector as previously described (Danko et al. 2008). Gas pressure was released using the Owen method (Owen et al., 1979) using a 20 or 50 mL glass syringe.

Production of volatile fatty acids (formate, acetate, propionate, *n*- and *i*-butyrate, valerate) and ethanol were determined using high pressure liquid chromatography (Jasco, Japan) using a Chrompack column (6.5 x 30 mm^2) with 0.7 mL/min sulfuric acid (0.005 mM) as the mobile phase. Detection was accomplished using a UV (210 nm) or refractive index detector (ethanol). The column temperature was set at 60 ºC.

Hydrogen production potential and rates were determined using the Modified Gompertz equation (Eq. 1) (Lay et al., 1999; Zwietering et al., 1990):

$$H(t) = P\,exp\left\{-exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\} \quad (1)$$

where:
$H(t)$ is the cumulative hydrogen production (mL)
P is the hydrogen production potential (mL),
R_m is the maximum hydrogen production rate (mL/hr)
λ is the duration of the lag phase (hr)
t is time (hr),
e is approximately 2.718.

3. Results and discussion

Hydrogen production occurred for both inoculum but there were differences in the amounts depending on the temperature or type of methanogenic inhibition (Fig. 1). Methane production was not detected in any of the batch experiments.

The Modified Gompertz equation was used to calculate the values for the maximum hydrogen production rate, hydrogen production potential, and duration of the lag phase for all batch reactors. In addition, the R^2 values listed are the ranges of the values obtained for modelling the individual triplicate bottles. The results are shown in Table 2.

BES-inhibited G and S produced in general more hydrogen at higher production potentials with smaller lag times when compared against heat treatment.

There was little difference in hydrogen production between BES and heat treatment for G at mesophilic temperatures (average difference of 1.9 mL H_2). However, the differences in the amounts of hydrogen production for heat and BES treatments were larger at thermophilic (8.3 mL H_2) and hyperthermophilic temperatures (3.1 mL H_2) for G.

The largest difference between the BES-inhibited biomass and the heat treated biomass was observed with the S inocula at 37 ºC where the hydrogen production by BES inhibition was three times larger than for the autoclaved inoculum.

Differences were also observed for hydrogen production rates and lag times. In general, BES inhibited biomass had higher production rates and smaller lag times when compared against heat treatment. The largest average difference in lag time (~ 66 hours) was observed for S at thermophilic temperatures.

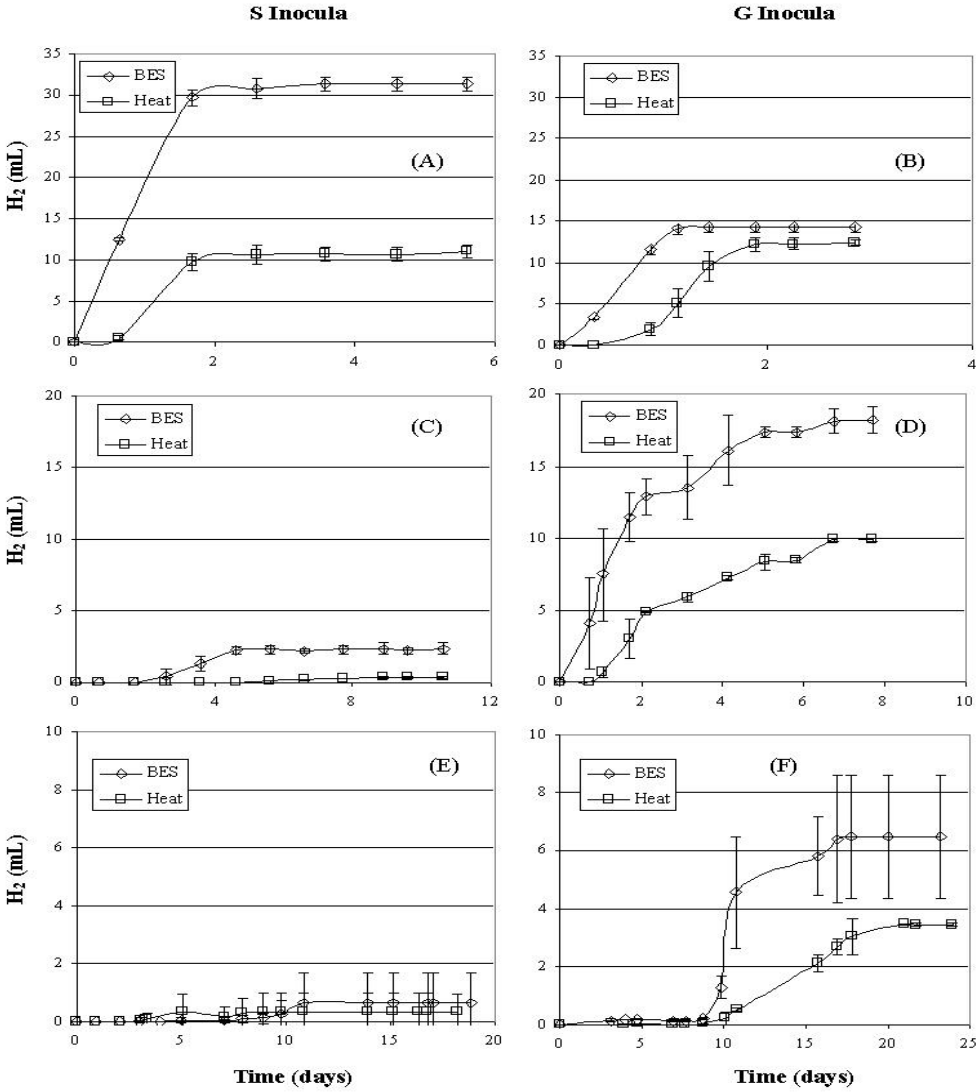

Fig. 1. Biohydrogen production from the S (Panels A, C, and E) and G (Panels B, D, and F) inocula at different temperatures and inhibition. The temperatures for the batch experiments were the following: 37 ºC for A and B, 60 ºC for C and D, and 70 ºC for E and F. Error bars represent one standard deviation of triplicate bottles.

Table 2. Modified Gompertz equation parameters for the two different sludges and two different methanogenic inhibitors where P = the hydrogen production potential, R_m = maximum hydrogen production rate, and λ = lag phase. The R^2 values listed are the range of the values obtained for modelling the individual triplicate bottles

Type of Inocula	Temperature (ºC)	Inhibition Treatment	P (mL)	R_m (mL/hr)	λ (hr)	R^2
S	37	Heat	11.23±0.15	0.64±0.23	14.96±1.82	0.9953-0.9980
		BES	31.39±0.87	1.99±1.08	9.01±2.67	0.9999-0.9999
G	37	Heat	12.51±0.39	0.73±0.14	20.21±1.40	0.9750-0.9949
		BES	14.38±0.54	0.86±0.06	4.30±0.52	0.9315-0.9989
S	60	Heat	0.38±0.12	0.01±0.00	128.80±21.96	0.9942-0.9969
		BES	2.29±0.29	0.09±0.06	63.39±22.83	0.9999-0.9999
G	60	Heat	9.65±0.03	0.11±0.01	16.02±6.19	0.9809-0.9896
		BES	16.98±0.99	0.35±0.11	3.64±1.65	0.9300-0.9976
S	70	Heat	ND*	ND	ND	ND
		BES	ND	ND	ND	ND
G	70	Heat	3.72±0.00	0.02±0.00	242.69±19.08	0.9993-0.9999
		BES	6.63±1.98	0.16±0.09	227.05±2.15	0.9933-0.9977

*ND means not determined

Previous studies have also indicated that heat treatment can be detrimental to hydrogen production when compared to other methods (Cheong and Hansen, 2006; Valdez-Vazquez et al., 2006; Zhu and Béland, 2006). G and S also showed significant differences for the effects of temperature. G was more robust with the effect of temperature for both BES and autoclaved biomass. Hydrogen production increased when the temperature was increased from 37 ºC to 60 ºC for BES inhibited sludge. However, hydrogen production potential decreased as the temperature increased from mesophilic to thermophilic temperatures.

As temperatures were increased a further 10 ºC from 60 ºC to 70 ºC, hydrogen production decreased approximately 55%. Similar results were observed when temperatures were increased from 50 to 55 ºC, although, hydrogen production and rates decreased only 25% (Lin et al., 2008).

For the S inocula, hydrogen production, potentials, and lag times were adversely affected as temperatures increased. Maximum hydrogen production for S was observed at 37 ºC (approximately 31 mL H_2) for BES-inhibition and decreased dramatically at 60 ºC (3 mL H_2). The amount of hydrogen produced from autoclaved S also decreased from 10 mL at 37 ºC to approximately 0.4 mL H_2 for 60 ºC. Hydrogen production was sporadic at hyperthermophilic conditions (70 ºC) as only one batch reactor (out of three) for each inhibition treatment produced hydrogen and therefore values for hydrogen production, potentials, and lag times were not determined for this experiment. Previous studies have shown that temperature can have a detrimental effect on hydrogen production (Lin et al., 2008; Valdez-Vazquez et al., 2006). This may be attributed to differences in microbial communities (Lin et al., 2008; Valdez-Vazquez et al., 2006).

The high degree of correlation between the data and the model for both inocula suggested that the Modified Gompertz Equation adequately described the data.

Results for the amount of sCOD, VFA, and ethanol production are shown in Table 3. Similar results on the effects of temperature and the type of methanogenic inhibition were observed with the amount of soluble COD (sCOD) and total VFA (VFA_{TOTAL}) that was produced for the G and S inocula. BES inhibited sludge produced on average more sCOD and VFA_{TOTAL} than the autoclaved sludge. In addition, the S inoculum was more adversely affected by the increases in temperature than was G, as was previously mentioned. For example, at 37 ºC, BES and autoclaved S inoculum produced 11600 and 6500 mg/L sCOD and 5800 and 3900 mg/L VFA_{TOTAL}, respectively. However, as the temperatures increased to 60 and 70 ºC, the amount of sCOD and VFA_{TOTAL} produced decreased significantly to levels less than 5400 mg/L and 2300 mg/L, respectively.

Also, the amount of sCOD and VFA_{TOTAL} produced was related to the amount of hydrogen that was generated. For example, the amount of sCOD and VFA_{TOTAL} was low (≤ 5500 mg/L and 2600 mg/L, respectively) when H_2 production was less than 6.5 mL. An increase in VFA_{TOTAL} was observed by Shin et al. (2004) when temperatures increased from mesophilic to thermophilic temperatures (Shin et al., 2004). However, Valdez-Vasquez et al. (2005) observed a decrease in VFA_{TOTAL} under the same temperature conditions (Valdez-Vazquez et al., 2006). Possible differences between the two studies may be attributed to differences in the microbial communities.

Table 3. Production of sCOD, H_2, and VFA during fermentation with two different inocula and two types of methanogenic inhibitors with food components

Type of Inocula	Temperature (ºC)	Inhibition Treatment	H_2 (mL)	sCOD (mg/L)	VFA_{TOTAL} (mg/L)	HFo (%)[a]	HAc (%)	HPr (%)	HBu (%)	EtOH (%)	HAc+HBu (%)
S	37	Heat	11.1±0.9	6 500	3 900	1.5%	33.1%	12.6%	52.5%	1.4%	85.6%
		BES	31.4±0.8	11 600	5 800	1.1%	36.1%	9.1%	52.9%	1.3%	89.0%
G	37	Heat	12.4±0.4	7 200	3 500	4.8%	20.2%	8.6%	63.0%	3.5%	83.2%
		BES	14.2±0.5	12 900	4 500	2.4%	26.6%	8.9%	58.7%	3.9%	85.3%
S	60	Heat	0.4±0.1	4 200	700	4.3%	35.7%	15.1%	41.3%	2.0%	77.0%
		BES	2.4±0.4	5 400	2 300	0.8%	27.7%	13.5%	56.1%	1.9%	83.8%
G	60	Heat	9.9±0.2	8 500	4 300	4.8%	26.4%	4.9%	57.9%	4.8%	84.3%
		BES	18.2±1.0	14 200	5 800	4.0%	27.7%	8.6%	55.8%	3.7%	83.5%
S	70	Heat	0.4±0.6	4 100	700	10.1%	46.2%	12.0%	30.9%	2.0%	77.1%
		BES	0.6±1.1	4 700	900	2.3%	42.8%	14.1%	44.6%	1.8%	87.4%
G	70	Heat	3.4±0.1	4 300	1 900	1.6%	16.1%	5.4%	74.0%	2.7%	90.1%
		BES	6.5±2.1	5 500	2 600	1.7%	22.9%	8.1%	63.1%	2.6%	86.0%

[a]Percentage of VFA_{TOTAL}

HFu, HAc, HPr HBu, EtOH stand for formate, acetate, propionate, butyrate, and ethanol, respectively.

Similarities and differences were also observed between S and G for individual VFAs. Formate and ethanol were generally the lowest percentage (as VFA_{TOTAL}) for both inoculum regardless of temperature. Propionate had the next highest percentage (average between 5 and 15% of VFA_{TOTAL}) but there were differences between the inocula. S inoculum produced HPr percentages generally above 10% (with the exception being autoclaved S at 37 ºC) while G was observed to produced HPr at values < 9% of VFA_{TOTAL}. The two largest amounts of VFA were from acetate and butyrate. This suggests that hydrogen is being produced *via* butyrate-acetate fermentation (Noike and Mizuno, 2000; Fang and Liu, 2002). The percentage of HAc and HBu was calculated to be between approximately 77 and 90% of VFA_{TOTAL} for both inocula. However, the percentage of acetate (as VFA_{TOTAL}) was generally higher in S compared to G. Conversely, S was also observed to have a lower percentage of HBu compared to G over the three temperature values. Differences in metabolic products at different temperatures have also been observed previously and may have been caused by different microbial communities (Lin et al. 2008; Shin et al. 2004).

4. Conclusions

The effect of methanogenic inhibitors, inoculum type, and temperature on biohydrogen production using food components was examined. In general, BES-inhibited sludge produced more hydrogen with higher rates and smaller lag times than heat treated suspended (S) and granular (G) inoculum. In addition, G was less sensitive to the effects of temperature as hydrogen production was observed for all three temperatures (37, 60, and 70 ºC) used with the maximum hydrogen production observed at 60 ºC. S was severely affected by temperature as hydrogen, sCOD, and VFA_{TOTAL} production all dramatically decreased as temperatures increased. Differences were also observed in individual VFAs. Differences in the microbial communities were likely responsible for the diverse behaviour of the two inocula.

Acknowledgements

We gratefully acknowledge the financial support awarded to Anthony Danko (SFRH/BPD/24221/2005) and to Ângela Abreu (SFRH/BD/29823/2006) through individual grants and the project (POCTI/ENR/57786/2004) from the Fundação para a Ciência e a Tecnologia (Portugal).

References

Abreu Â. A., Costa J. C., Araya-Kroff P., Ferreira E. C., Alves M. M., (2007), Quantitative image analysis as a diagnostic tool for identifying structural changes during a revival process of anaerobic granular sludge, *Water Res.*, **41**, 1473-1480.

Boyles D., (1984), *Bioenergy Technology - Thermodynamics and Cost*, New York, Wiley.

Cheong D.-Y., Hansen C. L., (2006), Bacterial stress enrichment enchances anaerobic hydrogen production in cattle manure sludge, *Appl. Microbiol. Biotechnol.,* **72**, 635-643.

Danko A. S., Abreu Â. A., Alves M. M., (2008), Effect of arabinose concentration on dark fermentation hydrogen production using different mixed cultures, *Int. J. Hydrogen Energy*, In press.

Das D., Veziroglu T. N., (2001), Hydrogen production by biological processes: a survey of literature, *Int. J. Hydrogen Energy*, **26**, 13-28.

Fang H. H., Liu H., (2002), Effect of pH on hydrogen production from glucose by a mixed culture, *Biores. Technol.,* **82**, 87-93.

Kim S.-H., Han S.-K., Shin H.-S., (2008), Optimization of continuous hydrogen fermentation of food waste as a function of solids retention time independent of hydraulic retention time, *Process Biochem.,* **43**, 213-218.

Kotay S. M., Das D., (2008), Biohydrogen as a renewable energy source - Prospects and potentials, *Int. J. Hydrogen Energy,* **33**, 258-263.

Kraemer J. T., Bagley D. M., (2007), Improving the yield from fermentative hydrogen production, *Biotechnol. Lett.,* **29**, 685-695.

Lay J.-J., Lee Y.-J., Noike T., (1999), Feasibility of biological hydrogen production from organic fraction of municipal solid waste, *Water Res.*, **33**, 2579-2586.

Levin D. B., Pitt L., Love M., (2004), Biohydrogen production: prospects and limitations to practical application, *Int. J. Hydrogen Energy*, **29**, 173-185.

Li C., Fang H. H. P., (2007), Fermentative hydrogen production from wastewater and solid wastes by mixed cultures, *Critical Reviews in Environmental Science and Technology*, **37**, 1-39.

Li S.-L., Kuo S.-C., Lin J.-S., Lee Z.-K., Wang Y.-H., Cheng S.-S., (2008), Process performance evaluation of intermittent–continuous stirred tank reactor for anaerobic hydrogen fermentation with kitchen waste, *Int. J. Hydrogen Energy*, **33**, 1522-1531.

Lin C.-Y., Chang R.-C., (2004), Fermentative hydrogen production at ambient temperature, *Int. J. Hydrogen Energy*, **29**, 715-720.

Lin C.-Y., Hung C.-H., Chen C.-H., Chung W.-T., Cheng L.-H., (2006), Effects of initial cultivation pH on fermentative hydrogen production from xylose using natual mixed cultures, *Process Biochem.*, **41**, 1383-1390.

Lin C.-Y., Wu C.-C., Hung C.-H., (2008), Temperature effects on fermentative hydrogen production from xylose using mixed anaerobic cultures, *Int. J. Hydrogen Energy*, **33**, 43-50

Liu D., Liu D., Zeng R. J., Angelidaki I., (2006), Hydrogen and methane production from household solid waste in the two-stage fermentation process, *Water Res.*, **40**, 2230-2236.

Morimoto M., Atsuko M., Atif A. A. Y., Ngan M. A., Fahhru'l-Razi A., Iyuke S. E., Bakir A. M., (2004), Biological production of hydrogen from glucose by natural anaerobic microflora, *Int. J. Hydrogen Energy*, **29**, 709-713.

Neves L., Gonçalo E., Oliveira R., Alves M. M., (2008), Influence of composition on the biomethanation potential of restaurant waste at mesophilic temperatures, *Waste Manage.*, **28**, 965-972.

Noike T., Mizuno, O., (2000), H_2 fermentation of organic municipal wastes, *Water Sci. Technol.*, **42**, 155-162.

Oh S.-E., Van Ginkel S., Logan B. E., (2003), The relative effectiveness of pH control and heat treatment for enhancing biohydrogen gas production, *Environ. Sci. Technol.*, **37**, 5186-5190.

Okamoto M., Miyahara T., Mizuno O., Noike T., (2000), Biological hydrogen production potential of materials characteristic of the organic fraction of municipal solid wastes, *Water Sci. Technol.*, **41**, 25-32.

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.

Shin H.-S., Youn J.-H., (2005), Conversion of food waste into hydrogen by thermophilic acidogenesis, *Biodegradation*, **16**, 33-44.

Shin H.-S., Youn J.-H., Kim S.-H., (2004), Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis, *Int. J. Hydrogen Energy*, **29**, 1355-1363.

Tchobanoglous G., Theisen H., Virgil S., (1993), *Integrated Solid Waste Management*, New York, McGraw-Hill, Inc.

Ueno Y., Tatara M., Fukui H., Makiuchi T., Goto M., Sode K., (2007), Production of hydrogen and methane from organic solid wastes from phase-separation of anaerobic processes, *Biores. Technol.*, **98**, 1861-1865.

Valdez-Vazquez, I., Ríos-Leal, E., Esparza-García, F., Poggi-Varaldo, H. M., (2005), Semi-continuous solid substrate anaerobic reactors for H_2 production from organic waste: mesophilic versus thermophilic regime. Int. J. Hydrogen Energy **30**: 1383-1391.

Valdez-Vazquez I., Ríos-Leal E., Muñoz-Páez K. M., Carmona-Martínez A., Poggi-Varaldo H. M., (2006), Effect of inhibition treatment, inocula, and incubation treatment on batch H_2 production from organic solid waste, *Biotechnol. Bioeng.*, **95**, 342-349.

Van Ginkel S., Sung S., Lay J.-J., (2001), Biohydrogen production as a function of pH and substrate concentration, *Environ. Sci. Technol.*, **35**, 4726-4730.

Yu H., Zhu Z., Hu W., Zhang H., (2002), Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures, *Int. J. Hydrogen Energy*, **27**, 1359-1365.

Zehnder A. J. B., Huser B. A., Brock T. D., Wuhrmann K., (1980), Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium, *Arch. Microbiol.*, **124**, 1-11.

Zhu H., Béland M., (2006), Evaluation of alternative methods of preparing hydrogen producing seeds from digested wastewater sludge, *Int. J. Hydrogen Energy*, **31**, 1980-1988.

Zwietering M. H., Jogenburger I., Rombouts F. M., Van ´T Riet, K., (1990), Modeling of the bacterial growth curve, *Appl. Environ. Microbiol.*, **56**, 1875-1881.