Effects of Heavy Metals and pH on the Conversion of Biomass to Hydrogen *via* Syngas Fermentation

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The effects of three heavy metals on hydrogen production *via* syngas fermentation were investigated within a metal concentration range of 0 to 1.5 mg Cu/L, 0 to 9 mg Zn/L, 0 to 42 mg Mn/L, in media with initial pH of 5, 6, and 7, at 55 °C. The results showed that at lower metal concentration, pH 6 was optimum while at higher metal concentrations, pH 5 stimulated the process. More specifically, the highest hydrogen production activity recorded was 155% ± 12% at a metal concentration of 0.04 mg Cu/L, 0.25 mg Zn/L, and 1.06 mg Mn/L and an initial medium pH of 6. At higher metal concentration (0.625 mg Cu/L, 3.75 mg Zn/L, and 17.5 mg Mn/L), only pH 5 was stimulating for the cells. The results showed that the addition of heavy metals, contained in gasification-derived ash, can improve the production rate and yield of fermentative hydrogen. This could lead to lower costs in gasification process and fermentative hydrogen production and less demand for syngas cleaning before syngas fermentation.

Keywords: Gasification; Syngas; Fermentative hydrogen; Heavy metals; pH

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INTRODUCTION

To face the increasing global waste generation and energy demand, sustainable strategies for the conversion of waste into green energy are required. Anaerobic fermentation is a relatively simple and cost-effective method that converts organic wastes, such as food residuals, sewage waste, and manure, into biofuels. However, there is a big fraction of solid waste, such as forest residues and mixed landfill wastes, which are difficult or impossible to degrade biologically because of their complex structure. An effective way to treat this recalcitrant biomass is with the combination of gasification and anaerobic fermentation processes. During this two-stage process, the feedstock is converted into syngas, a gas consisting mainly of hydrogen, carbon monoxide, and carbon dioxide. Thereafter, anaerobic bacteria digest the syngas and create value-added chemicals, such as volatile fatty acids (VFA), and biofuels such as hydrogen.

Hydrogen is a valuable gas with a high-energy content of 120 MJ/kg to 142 MJ/kg, which is higher than that of hydrocarbon fuels by a factor of approximately 2.75 and releases only water during combustion (Henstra *et al.* 2007; Tufa *et al.* 2016). This gas has numerous applications in chemical processes, lamps, balloons, vehicle fuels, laboratories, as a reductive agent (redox reactions), *etc*. Hydrogen is also a product of several processes. The largest fraction of the global hydrogen production (80% to 85%) is obtained by the steam methane reforming (SMR) process of natural gas (Fan *et al.* 2016), which uses fossil fuels as the main substrate. In addition, electricity can be stored in hydrogen form through the power-to-gas process. Another process for hydrogen generation, with less environmental drawbacks but higher costs, is the water electrolysis method, although it currently can only be used in small-scale applications (Tufa *et al.* 2016). Biocatalysts, such as microalgae and phototrophic bacteria, have been employed in special-designed photobioreactors for hydrogen production (Skjånes *et al.* 2016). Another biological process is the dark fermentation of biomass such as food residues, manure, and straw. The main advantage of the fermentation process for hydrogen production is that it is less expensive, less energy-demanding, and more eco-friendly than the other conventional processes (Levin *et al.* 2004; Lin and Shei 2008). All the above processes can lead to a substantial production of hydrogen to be used for energy and as material resources.

The anaerobic syngas conversion into hydrogen can occur *via* a biological water gas shift process. In this process, carbon monoxide acts as an electron donor and the CO-dehydrogenase (CODH) enzyme provides electrons and protons for the carbon monoxide. Thereafter, the electrons released by the oxidation reaction are transferred to another enzyme called energy-converting hydrogenase (ECH) (Henstra *et al.* 2007), which catalyzes the reduction of electrons for the production of molecular hydrogen. Fatty acids are by-products that can be produced through the H_2/CO_2 or CO consumption pathway (Grimalt-Alemany *et al.* 2017), shown in Eq. 1,

$$CO + H_2O \rightarrow CO_2 + H_2, \Delta G^0 = -20 \text{ KJ/mol} \quad (1)$$

At the end of the gasification process, inorganic components, such as heavy metals, still remain in the residual ashes (Dong *et al.* 2015). These metals can be present in different forms such as metal oxides or metal chlorides (Liao *et al.* 2007; Tafur-Marinos *et al.* 2014), and their composition depends on the feedstock and gasification conditions (Dong *et al.* 2015). Despite the fact that the gasification ashes have been used as building materials for roads and other constructions, they can leach and pose a threat to the environment and to the public's health (James *et al.* 2012). The main threat derives from the heavy metals, which are toxic and classified as known or possible carcinogens for humans, according to the U.S. Environmental Protection Agency and the International Agency for Research on Cancer (Tchounwou *et al.* 2012). In contrast, heavy metals have been shown to be beneficial for the growth of fermentative microbes (Osuna *et al.* 2003). For instance, a study on fermentative hydrogen production reported that the addition of Cu and Cr ions improved the hydrogen yield (Lin and Shei 2008). Therefore, the use of heavy metals during syngas fermentation could reduce the environmental footprint of gasification while enhancing the fermentation process.

Although several studies have been conducted on the effects of heavy metals on anaerobic methane production (Fang and Chan 1997; Lin and Chen 1999; Abdel-Shafy and Mansour 2014), there are only a handful of studies on the effects of heavy metals on the fermentative hydrogen production (Li and Fang 2007; Lin and Shei 2008). According to the authors' best knowledge, this is the first study that investigates the impact of heavy metals in syngas fermentation. The aim of this work is to provide valuable data on the beneficial and inhibitory effects of heavy metals and pH during syngas fermentation and suggest strategies for improving the efficacy of this process. This knowledge can be used in order to reduce the environmental impact of gasification and reduce the costs of syngas cleaning and fermentative hydrogen production.

EXPERIMENTAL

Materials

Liquid medium

The liquid medium inside the reactors consisted of micronutrients in trace amounts, macronutrients, and the three heavy metals that were under investigation (except the control reactors).

The concentration of the inorganic macronutrients in the used liquid medium was 280 mg NH_4Cl/L, 330 mg K_2HPO_4•$3H_2O$/L, 100 mg MgSO4•$7H_2O$/L, and 10 mg $CaCl_2$•$2H_2O$/L (Osuna *et al.* 2003). Moreover, 1 mL of the trace element stock solution was contained in 1 L of the liquid medium. The concentration of the trace element stock solution included 2000 mg $FeCl_2$•$4H_2O$/L, 50 mg H_3BO_3/L, 50 mg $ZnCl_2$/L, 500 mg $MnCl_2$•$4H_2O$/L, 38 mg $CuCl_2$•$2H_2O$/L, 50 mg $(NH_4)_6Mo_7O_{24}$•$4H_2O$/L, 2000 mg $CoCl_2$•$6H_2O$/L, 142 mg $NiCl_2$•$6H_2O$/L, and 164 mg Na_2SeO_3•$5H_2O$/L (Osuna *et al.* 2003).

The metal ions used were in the form of metal oxides (ZnO, CuO) and salt ($MnCl_2$•$4H_2O$) powder. Initially, 0.25 g of each metal powder was dissolved in 20 mL of 2% nitric acid (HNO_3), and deionized water was added to achieve a total volume of 500 mL. The metal stock solutions were dissolved to achieve a metal ratio (Cu:Zn:Mn) of approximately 1:6:28. This metal ratio is similar to the heavy metal composition that was found in the fly and bottom ashes of a wood pellet pyro-gasification facility (Tafur-Marinos *et al.* 2014).

Syngas composition

A commercial syngas mixture containing carbon monoxide (55 mol%), hydrogen (20 mol%), and carbon dioxide (10 mol%) (Klasson *et al.* 1990) was provided by AGA Gas AB (Gothenburg, Sweden). The overpressure in the gas cylinder containing the syngas was built by nitrogen gas.

Inoculum

The mixed anaerobic consortium was obtained from a local thermophilic 3000 m^3 anaerobic digester that typically digested the organic fraction of municipal solid waste (Borås Energy and Environment, Borås, Sweden). A typical pH value in the digester was 8 to 8.2 and the main fraction of the substrate was food waste. The total solids (% TS) and volatile solids (% VS) of the inoculum were 14.23% ± 0.27% and 14.15% ± 0.27%, respectively.

Methods

Reactor characteristics, inoculation, and start-up

The reactors were serum glass bottles with plastic caps, rubber sealing, and a total volume of 118 mL (Bioprocess Control AB, Lund, Sweden). The anaerobic culture was incubated at 55 °C for 5 days to consume all the nutrients prior to the experiment. After incubation, the excess water from the inoculum was removed by centrifugation at 4300 ×*g* for 10 min. Then, each reactor was loaded with 3 g of inoculum and filled with 40 mL of liquid medium. The headspace of each reactor was purged with syngas for 3 min with a flow rate of 50 mL/min in order to remove air and feed the reactor with the gaseous substrate. After the syngas-purging, the pressure inside the headspace was adjusted at 1 atm with a needle that removed the excess gas. The reactors were continuously shaken (100 rpm) in a warm water bath (55 °C ± 1 °C) and placed at a 45° angle. The gaseous samples were collected from the top of the reactors through the rubber sealing regularly, and the liquid samples were collected at the end of the experiment from the liquid medium.

The inoculum was acclimated to syngas as the sole carbon and energy source for 15 days before the experimental results were obtained. The experiment was conducted in batch mode and in triplicates, and the results are presented as the mean values ± standard deviation. The duration of the experiment was 96 h.

Gas analysis

Gas samples were collected from the headspace of the bioreactors every 12 h with a 0.25-mL gas-tight syringe (VICI, Precision Sampling Inc., Baton Rouge, LA, USA). The gas components were analyzed by using gas chromatography (GC). For the analysis of hydrogen and carbon dioxide, a Perkin-Elmer gas chromatograph (Clarus 500; Norwalk, CT, USA) was used, equipped with a packed column (CarboxenTM 1000, 6’ × 1.8” OD, 60/80 Mesh, Supelco, Shelton, CT, USA) using a thermal conductivity detector (Perkin-Elmer, Norwalk, CT, USA) with an injection temperature of 200 °C. The levels of the carbon monoxide were also analyzed by another gas chromatograph (Clarus 400; Perkin-Elmer, Norwalk, CT, USA), equipped with a packed column (CarboxenTM 1000, 6’ × 1.8” OD, 60/80 Mesh, Supelco, Shelton, CT, USA) and a thermal conductivity detector (Perkin-Elmer, Norwalk, CT, USA) with an injection temperature of 200 °C. Both of the gas chromatographs used nitrogen as a carrier gas with a flow rate of 30 mL/min at 75 °C.

Liquid analysis

The VFA content in the liquid samples was analyzed using a Waters 2695 high-performance liquid chromatograph (HPLC; Waters Corporation, Milford, CT, USA) with a hydrogen-based column (Aminex HPX87-H; BioRad Laboratories, München, Germany) at 60 °C and 0.6 mL/min (5 mM H_2SO_4 eluent) equipped with a refractive index (RI) detector (Waters 2410, Waters Corporation, Milford, CT, USA). The metal concentrations of the liquid samples were measured with a microwave plasma-atomic emission spectrometer (MP-AES 4200; Agilent Technologies, Santa Clara, CA, USA) equipped with an inlet air filter kit - 4107 nitrogen generator (Agilent Technologies, Santa Clara, CA, USA) and an SPS 3 Autosampler (Agilent Technologies, Santa Clara, CA, USA).

RESULTS AND DISCUSSION

The coupling of thermochemical and biochemical processes, such as gasification and dark fermentation, can convert recalcitrant biomass into hydrogen. The use of heavy metals derived from the ashes of thermochemical processes can reduce the environmental footprint of these processes and boost the efficacy of biochemical processes.

This work focused on the biochemical conversion of syngas into hydrogen through dark fermentation. The goal was to investigate the effect of heavy metals and pH and to report the optimum, inhibiting, and toxic metal concentrations and pH values. Hydrogen production activity (Eq. 2), hydrogen yield (Eq. 3), and total VFA yield (Eq. 4) were employed to monitor the extent of heavy metal and pH effects on the syngas fermentation process. The heavy metal uptake (%), was also obtained. Equation 2 shows the hydrogen production activity (%),

$$A_H\ (\%) = (A_i / A_c) \times 100 \tag{2}$$

where A_c is the hydrogen amount (mmol) produced by the control reactors, and A_i is the hydrogen amount (mmol) produced by the metal-dosed reactors after 96 h of anaerobic fermentation. The yield values were calculated as follows,

$$Y_H = H_2 \text{ produced / CO fed} \tag{3}$$

$$Y_{VFA} = \text{VFA concentration / CO fed} \tag{4}$$

where Y_H is the hydrogen yield (mmol H_2/mmol CO fed) and Y_{VFA} is the VFA yield (g VFA/L/mmol CO fed) obtained after 96 h of anaerobic fermentation.

The Effect of HNO_3

The addition of HNO_3 in the reactors had two main purposes. First, the acid dissolved the heavy metal compounds inside the liquid medium and, second, the addition of HNO_3 shifted the biological activity from methane to hydrogen production. Thus, no inoculum pretreatment that favored hydrogen production and the inhibition of methanogens was necessary. Figure 1 (panels *a* through *d*) shows how the consumption/production of gas components was affected by the addition of HNO_3. The HNO_3 concentration that inhibited methane production was 0.08%. This nitrate concentration was relatively low in comparison to the concentration used in other studies. For example, in a work that investigated the effect of ammonia and nitrate on biogas production from food waste, no inhibitory effect on methane production was reported at total ammonia nitrate addition levels below 1.1 g/L (Sheng *et al.* 2013). However, these inhibitory levels depend also on the anaerobic species of the inoculum.

Several mechanisms have been proposed to explain the inhibition of methanogens by HNO_3. One possible mechanism is that nitrate raises the redox potential, E_h, of the medium (Jones 1972). Another reason may be the toxic effects of nitrogen intermediates such as nitrite and nitrous oxide (Iwamoto *et al.* 1999; Ungerfeld 2015). Recent studies suggest that nitrate can be used as an alternative hydrogen sink so that it can compete with available hydrogen, which is one of the main methane substrates together with carbon dioxide (Yang *et al.* 2016). However, in this work, hydrogen production was favored, and therefore it can be assumed that the inhibiting mechanism was based on the toxicity of nitrate on methanogens.

The Effect of Heavy Metals and pH

Syngas fermentation for hydrogen production took place in bioreactors containing media with heavy metal concentrations between 0 mg Cu/L, 0 mg Zn/L, 0 mg Mn/L, and 1.5 mg Cu/L, 9 mg Zn/L, 42 mg Mn/L and the different initial medium pHs that were investigated were 7, 6, and 5. The metals were added in a specific ratio in the medium to mimic the real metal composition in the ash of a wood pellet gasification plant as described previously.

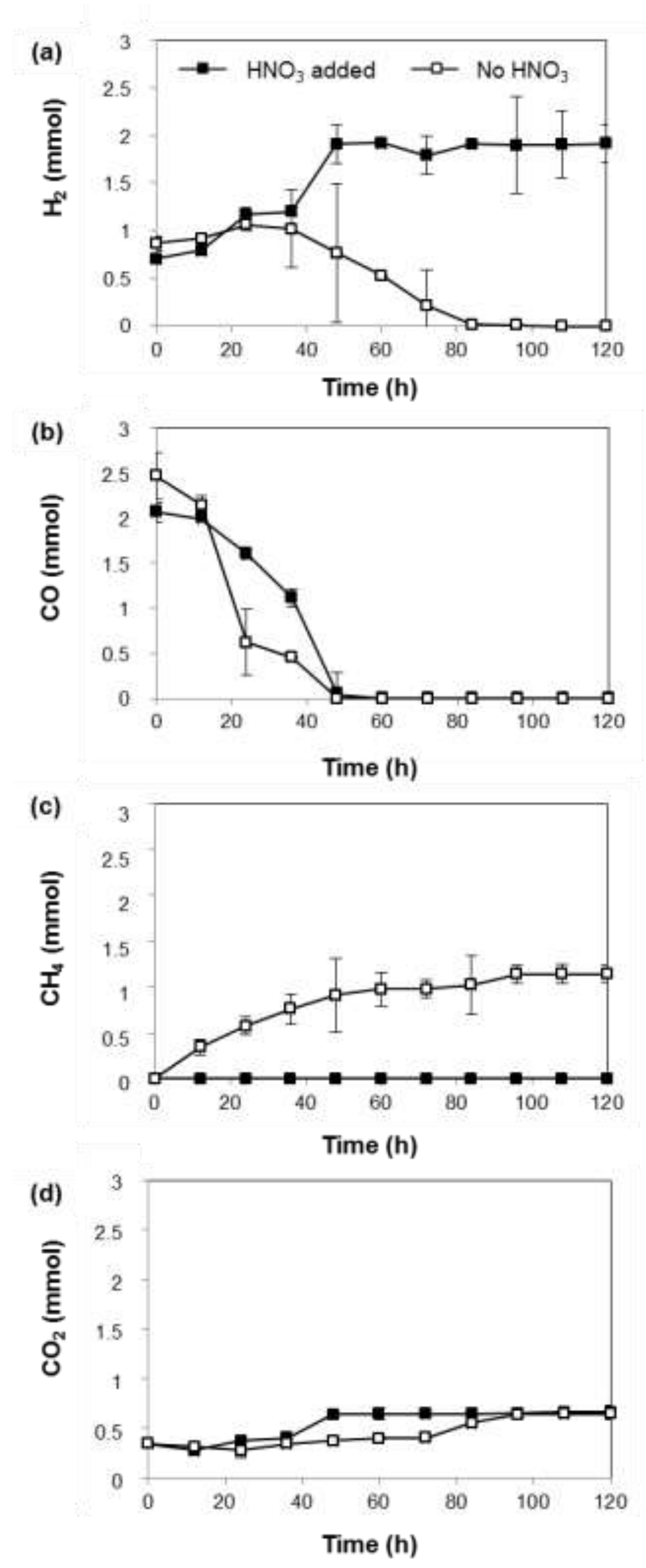

Fig. 1. Effect of the addition of HNO_3 during syngas fermentation

Figure 2 shows the relationships between the accumulated hydrogen production, heavy metal concentrations, and initial pH of the medium.

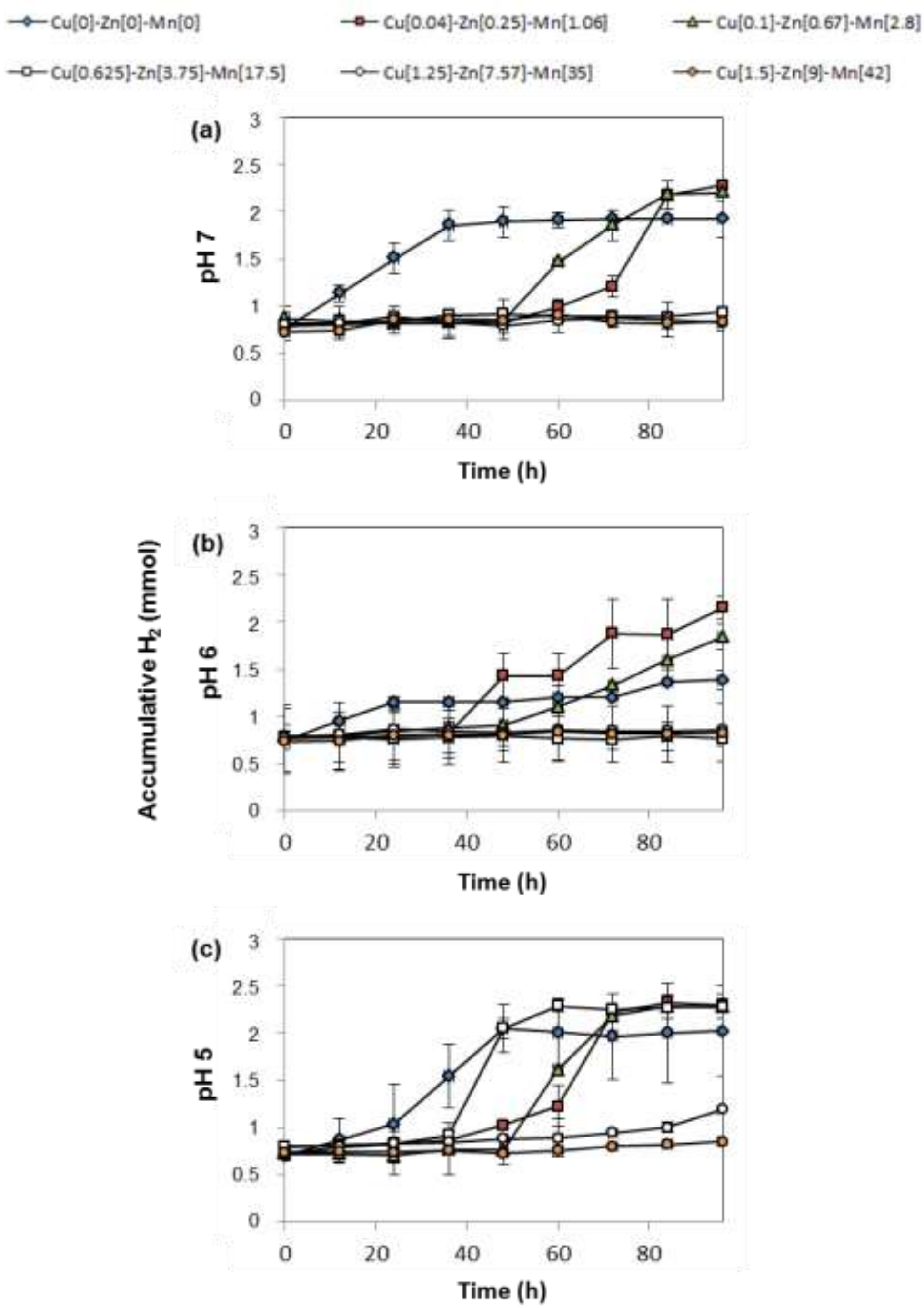

Fig. 2. Relationships between accumulative hydrogen production, initial medium pH of 7 (a), 6 (b), and 7 (c), and heavy metal concentrations (mg/L)

The accumulative hydrogen amount was calculated by the total biogas production and hydrogen gas content. Figure 2 also shows the time (h) that was required for each bioreactor to reach its maximum hydrogen production in 96 h of fermentation. The results showed that the different heavy metal loadings and the initial pHs of the medium influenced the hydrogen production.

The role of heavy metals in anaerobic fermentation is essential, as many of them are part of the enzymes as metallic co-factors. More specifically, Mn stabilizes the enzyme methyltransferase (MPB) and affects redox reactions (Fisher *et al.* 1973; Perry and Silver 1982), Cu affects the superoxide dismutase (SODM) and hydrogenase in MPB in *Clostridia* and facultative anaerobes (Jones *et al.* 1987; Kirby *et al.* 1981), and Zn affects the activity of hydrogenase in MPB, the sulfate-reducing bacteria (SRB), and formate dehydrogenase (FDH) (Adams *et al.* 1986; Kirby *et al.* 1981). A total lack of or overdose of heavy metals may cause the inhibition or loss of enzyme functions. Usually, at high concentrations, metals act as nonspecific, reversible inhibitors and do not compete with the substrate. In this type of inhibition, the metals bind to enzymes and form the enzyme-inhibitor (EI) or enzyme-substrate-inhibitor (ESI) complexes of EI or ESI type (Wood and Wang 1983). However, some heavy metals, such as Zn and Cd, may cause competitive inhibition, meaning that they may compete with the substrate. Possible microbial inhibition mechanisms include substitution of the metallic enzyme co-factors, combining with the sulfhydryl group (-SH), and tight binding to acid groups in the polypeptide chains (Wood and Wang 1983).

The hydrogen production activity (A_H) and hydrogen yield (Y_H) are shown in Fig. 3. The reactors dosed with low heavy metal concentrations showed a higher hydrogen production in comparison to the control reactors. In particular, the metal concentrations of 0.04 mg Cu/L, 0.25 mg Zn/L, and 1.06 mg Mn/L and 0.1 mg Cu/L, 0.67 mg Zn/L, and 2.8 mg Mn/L stimulated the hydrogen production activity (A_h > 100%). The highest hydrogen production activity recorded was 155% ± 12% at a metal concentration of 0.04 mg Cu/L, 0.25 mg Zn/L, and 1.06 mg Mn/L and initial medium pH 6. Another study reported that a metal concentration of 3 mg Cu/L resulted in a 10% to 20% increase in hydrogen production (Lin and Shei 2008). Other studies on fermentative hydrogen production also have shown that the highest hydrogen production was achieved in mediums with a pH of 6 and that lowering the pH inhibited hydrogen formation (Dareioti *et al.* 2014). This phenomenon depends on the type of the dominant bacterial species inside the anaerobic microbial community of the inoculum. For example, *Clostridia*, which are usually dominant in this process, function within a pH range of 6.0 to 6.7. The pH is a very crucial parameter for the microbial growth of anaerobic communities and it directly affects the fermentative hydrogen production. The pH of the medium changes the intracellular pH and the electrochemical gradient across the microbial membrane and thus influences the regulation of metabolism and the bioenergetics of microorganisms.

Metal concentrations higher than 0.1 mg Cu/L, 0.67 mg Zn/L, and 2.8 mg Mn/L were demonstrated to be inhibitory for the cells in media with pH 6 and 7 (Fig. 3). More specifically, media containing metal mixtures of 1.25 mg Cu/L, 7.57 mg Zn/L, and 35 mg Mn/L and 1.5 mg Cu/L, 9 mg Zn/L, and 42 mg Mn/L were shown to be inhibitory and reduced the hydrogen production activity to values as low as 35%. Another study reported a 50% reduction in the hydrogen production activity for mixed inoculum that was dosed with media containing 4.5 mg Zn/L and 6.5 mg Cu/L (Lin and Shei 2008). These concentrations are higher but comparable to the inhibiting metal concentrations found in this study. However, the metal concentrations of 0.625 mg Cu/L, 3.75 mg Zn/L, and 17.5 mg Mn/L stimulated the hydrogen production activity in mediums with a pH of 5. Therefore, for substrates with higher metal concentrations, pH 5 is recommended. This shift in the preference of pH at higher metal concentrations is possibly connected to the types of different strains of the microbial community and their optimum conditions of growth.

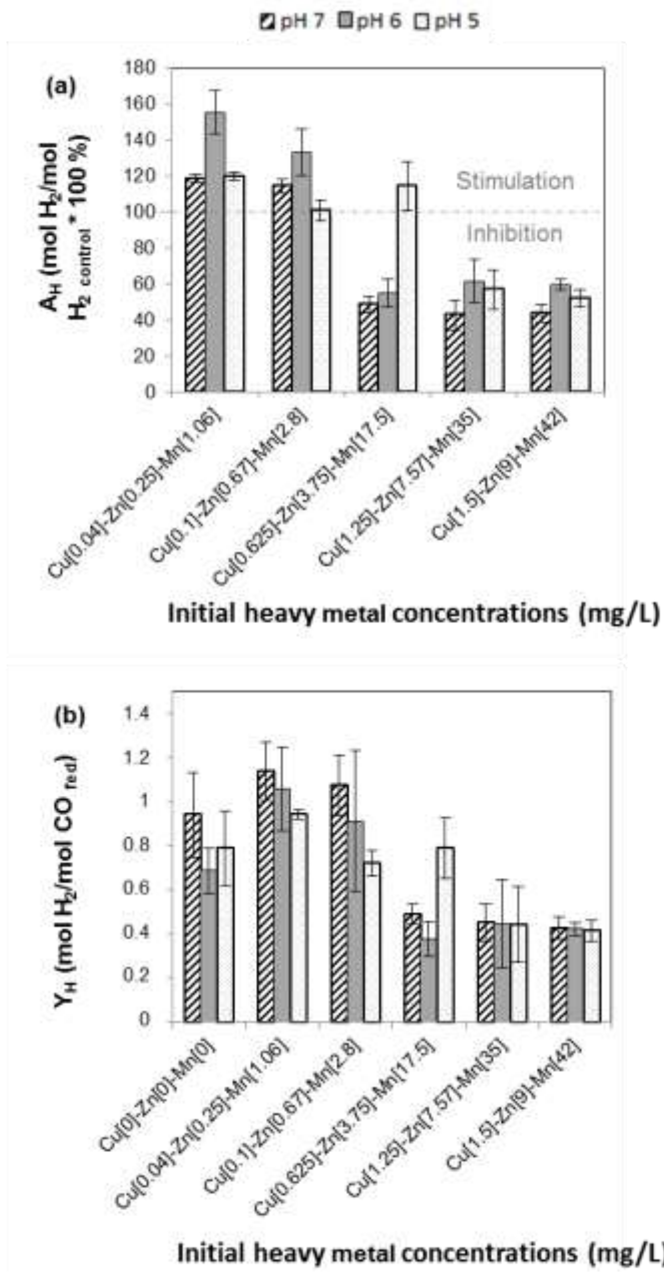

Fig. 3. Relationships between hydrogen production activity, A_h (%) = (mol H_2/mol H_2 control)*100 (a), hydrogen yield, Y_H = mol H_2 prod./mol CO fed (b), initial medium pH and heavy metal concentrations (mg/L)

The hydrogen yield showed a similar result to the hydrogen production activity (Fig. 3). The hydrogen yields were stimulated at lower metal concentrations and inhibited at higher metal concentrations. The highest hydrogen yield achieved was 1.32 mol ± 0.02 mol H_2 prod./mol CO fed in bioreactors containing 0.1 mg Cu/L, 0.67 mg Zn/L, and 2.8 mg Mn/L and medium with initial pH 7.

Volatile fatty acid production was detected only in the control reactors (Fig. 4). This meant that the presence of heavy metals inhibited complete acidogenesis even though hydrogen formation was favored at low metal concentrations (Fig. 3). The VFA formation increased as pH decreased and the highest VFA yield reported was 1.086 g VFA/L per mmol of carbon monoxide fed, at a pH of 5.

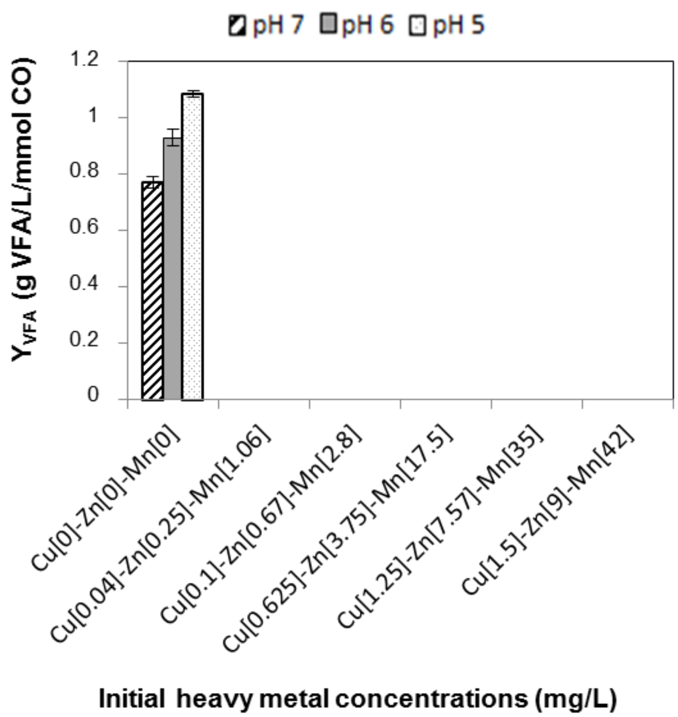

Fig. 4. Relationships between VFA yield, Y_{VFA} = g_{VFA}/L/mmol CO fed, initial medium pH, and heavy metal concentrations (mg/L)

Heavy Metal Uptake (%)

During anaerobic fermentation, the heavy metals can end up inside the cells or they can be absorbed or precipitated with other inorganics (Hayes and Theis 1978; Howgrave-Graham and Wallis 1991). The availability of heavy metals as nutrients or as inhibitors is affected by the concentration of the metals as well as other parameters such as the pH of the liquid medium (Oleszkiewicz and Sharma 1990). However, even if there is a full availability of the metals as nutrients, this does not mean that the microorganisms utilize the metals. The metal uptake may be hindered by the presence of other metals, the lack of carrier molecules inside the cells, high excretion of metal ions by the cells, and the failure of the energy driven system (Wood and Wang 1983). In the case of the opposite conditions, excessive metal uptake may take place. In general, the uptake of heavy metals and all other nutrients indicate the growth rate of the cells (Oleszkiewicz and Sharma 1990).

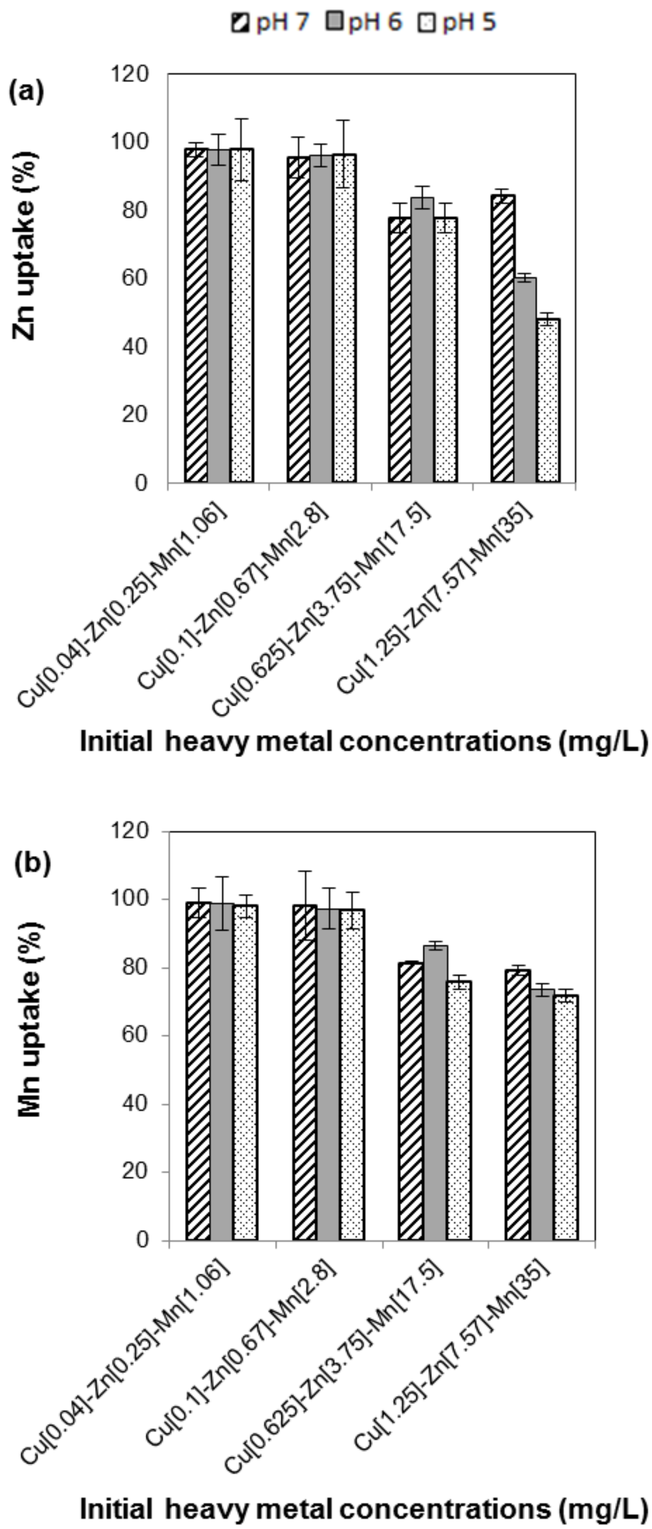

Fig. 5. Relationships between metal uptake (%) of Zn (a) and Mn (b), initial medium pH, and initial heavy metal concentrations (mg/L)

In this work, the metal uptake was calculated from the initial and final metal concentrations inside the liquid medium of the reactors (Fig. 5). The results showed that the uptake of Zn and Mn was approximately 100% up to a metal concentration of 0.1 mg Cu/L, 0.67 mg Zn/L, and 2.8 mg Mn/L. At a metal concentration of 0.625 mg Cu/L, 3.75 mg Zn/L, and 17.5 mg Mn/L the metal uptake decreased to 90–80%, while at a higher metal concentration of 1.25 mg Cu/L, 7.57 mg Zn/L, and 35 mg Mn/L the Zn and Mn uptake dropped especially in media with lower pHs. This result was similar to the findings of another study that investigated the uptake of several heavy metals, at different pHs, during anaerobic digestion (Wang *et al.* 2007). That study also concluded that heavy metal uptake increased at higher pH values. In addition, several studies have shown that low pHs may hinder the heavy metal uptake (Cheng *et al.* 1975; Wang *et al.* 1999) because of the pH value's influence on the binding of metals on the microbial cell wall (Wang *et al.* 2007). However, the pH inhibition level also depended on the type of each metal. In studies performed with activated sludge, it was reported that the main binding sites of the metals on the sludge sites were amino acids on the cell wall. Some metals, such as Cu and Pb, exhibit a strong affinity for anaerobic sludge sites (Artola *et al.* 1997), while other metals, such as Zn and Ni, show a relatively low affinity (Leighton and Forster 1997; Artola *et al.* 2000).

The above results showed that the addition of heavy metals during syngas fermentation improved hydrogen production. In addition, the appropriate combination of metal concentrations and initial pH of the liquid medium increased the efficacy of the process.

CONCLUSIONS

1. The addition of Cu, Zn, and Mn improved hydrogen production during syngas fermentation.
2. The highest hydrogen production activity achieved was 155% ± 12% at a metal concentration of 0.04 mg Cu/L, 0.25 mg Zn/L, and 1.06 mg Mn/L and a pH of 6.
3. At a higher metal concentration of 0.625 mg Cu/L, 3.75 mg Zn/L, and 17.5 mg Mn/L, pH 5 was optimum.
4. The uptake of Zn and Mn decreased at higher metal doses and lower pHs.

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