

# Anaerobic Digestion of Residual Lignocellulosic Materials to Biogas and Biohydrogen

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*Arundo donax* (giant reed) hydrolysate was exploited as a substrate for anaerobic digestion aimed to the production of biogas and biohydrogen. A mixed culture adapted from a primary sludge digester was used as inoculum. Besides the biogas, the anaerobic fermentation products were ethanol, acetic acid and butyric acid. Together, the soluble products accounted for about 51 % of degraded carbon. The produced biogas was 5 % H<sub>2</sub>, 41 % CO<sub>2</sub> and 54 % CH<sub>4</sub> by volume. Fermentation kinetics was slower and biogas yields were lower than those found with glucose fermentation with same mixed culture. Further optimization of the process is envisaged in order to improve biohydrogen yield.

## 1. Introduction

Hydrogen is predicted as the main energy carrier of the future (Mazloomi and Gomes, 2012). As a matter of facts, it has a higher energy yield in comparison to fossil fuels, and can be used to produce directly energy by fuel cells. In addition, it is abundantly available in the universe, odorless, non poisonous, and its combustion does not generate pollutants (Pant and Gupta, 2009). Nevertheless, the diffusion of the hydrogen as a fuel is still limited by the higher costs of production and storage.

The biological production offers a possible approach to obtain a sustainable supply of hydrogen (Hallenbeck et al., 2012). The biological production of H<sub>2</sub> could in principle be based on the direct exploitation of the photosynthetic activity of algae (biophotolysis) or bacteria (photofermentation) (Kapdan and Kargi, 2006). Yet, the development of these processes is still limited by their reduced photosynthetic efficiencies, as well as to the oxygen-related inhibition of the hydrogenase enzymes, which act as proton-activating catalysts in the biophotolysis. Consequently, a practical application of these methods will likely require a long term (Hallenbeck et al., 2012).

Currently, greater potentialities are offered by the microbial fermentative production of hydrogen, also called Dark Fermentation (DF), for different reasons (Chong et al., 2009):

- DF is not subject to the limitations associated to photosynthesis, as it is carried out under anaerobic conditions, thus avoiding the hydrogenase inhibition, and does not require the direct capture of solar energy;
- DF can be carried out using a technology similar to that of methanogenesis by anaerobic digestion, widely employed at industrial scale and consequently easier to be developed in the short term at industrial level;
- DF allows the exploitation of a wide variety of potential substrates, either wastes or biomasses, that are largely available at reduced costs.

The development of an efficient technology to obtain renewable energy from lignocellulosic biomasses could open new perspectives as regards the hydrogen production, as a large range of agroforestry wastes can be recycled, such as non-food parts of crops (stems, leaves and husks), forest products, and also industry wastes (woodchips, skin and pulp from fruit pressing, etc.). So far, much attention has been

directed towards the exploitation of lignocellulosic biomasses for the production of bioethanol. In the last years, different papers have been concerned at the DF of lignocellulosic feedstocks (Saratale et al., 2008), such as sorghum (Antonopoulou et al., 2008), poplar leaves (Cui et al., 2010), miscanthus (de Vrije et al., 2009), cornstalks (Lu et al., 2009), wheat straws (Nasirian, 2012), and bagasse obtained from sugar extraction processes (Chairattananokorn et al., 2009). The economic feasibility of small and medium scale applications is expected to play a crucial role in the future, due to their flexibility and adaptability to local availability of feedstocks (Karaoglanoglou et al., 2012).

This study is aimed to produce biogas and biohydrogen by anaerobic digestion of *Arundo donax* hydrolysates with a mixed culture. *A. donax* was chosen as a source of lignocellulosic biomass because it is a perennial crop particularly suitable for energy production owing to biomass yields as high as 37.7 t of dry matter per ha and per year (Angelini et al., 2009). Being a non-food crop, it can be cultivated in partially fertile or polluted soils, not used for agriculture. The use of a naturally occurring mixed culture was preferred to the use of selected strains in view of the application of the technique to non-sterile reactor operation.

## 2. Materials and Methods

### 2.1 Synthetic medium fermentations

Sewage sludge was obtained from a primary sludge digester of a municipal wastewater treatment plant (Nola, Italy). The anaerobic consortium was adapted to a synthetic medium by performing several transplants.

Synthetic medium contained M9 salts ( $\text{Na}_2\text{HPO}_4$  7.0 g/L,  $\text{KH}_2\text{PO}_4$  3.0 g/L, NaCl 0.5 g/L,  $\text{NH}_4\text{Cl}$  1.0 g/L), supplemented with glucose 10 g/L and trace elements. Resazurin (0.025%) was also added as anaerobiosis indicator.

Fermentation was carried out in 125 mL clamped Pyrex vials with perforable butyl rubber septa. Each vial was filled with 80 mL of concentrated culture medium, and inoculated with 20 mL of the anaerobic consortium. Anaerobic conditions were ensured by sparging the medium with nitrogen gas. The vials were kept at 35°C and 160 rpm in a basculating incubator (Infors HT Minitron).

### 2.2 *Arundo donax* hydrolysate fermentations

Giant Reeds (*Arundo donax*) were collected from Torre Lama (Campania, Italy) agro-land. Leaves were separated from stems, washed, dried overnight at 80°C and grind with a chopper. The powder was stored in desiccators. In a typical hydrolysis procedure, 20 g of oven-dried powder were added to 200 mL of a 5% (w/v) HCl solution, and autoclaved at 121°C for 20 min. After filtration (with filter paper), the pH was adjusted to 6.5 by adding a NaOH solution.

*Arundo donax* hydrolysate was supplemented with M9 salts and trace elements. Resazurin (0.025%) was also added as anaerobiosis indicator. Final concentration of total sugars in fermentation medium was about 10 g/L.

Fermentation tests with hydrolysates were carried out following the same procedure adopted with synthetic medium.

### 2.3 Analytical techniques

Sampling of liquid and gaseous phases from clamped vials was performed according to standard anaerobic techniques (Strobel, 2009).

The biomass concentration was monitored by measuring turbidity of liquid samples at 600 nm. After centrifugation and filtration with 0.2 µm cut-off filters, the liquid sample was analysed for residual substrate content (glucose or total sugars) and soluble fermentation products (volatile acids, alcohols).

The concentration of glucose was measured following a modified Nelson-Somogyi method for reducing sugars (Nelson, 1944). Total sugars were measured according to the phenol/sulphuric method (DuBois et al., 1956).

Concentration of volatile acids (acetic acid, butyric acid) and ethanol was determined by GC analysis, using a Shimadzu GC-17A equipped with a FID detector and a capillary column with a PEG stationary phase (BP20, 30 m by 0.32 mm i.d., 0.25 µm film thickness, from SGE). 1 µL samples were injected with a split-ratio of 1:10. Helium was fed as carrier gas with a flow rate of 6.5 mL/min. Injector and detector temperatures have been set to 320 °C and 250 °C, respectively. Initial column temperature has been set to 30 °C, kept for 3 min, followed by a ramp of 10 °C/min till 140 °C, kept for 1 min.

Gas ( $\text{H}_2$ ,  $\text{CO}_2$ , and  $\text{CH}_4$ ) composition was determined by GC analysis, using a HP 5890 equipped with a TCD detector and a molecular sieve capillary column. 0.2 mL samples were injected with a gas-tight

syringe. The temperatures of column, injector and detector were kept at 60 °C. Helium was used as carrier gas at pressure of 300 kPa.

## 2.4 Biogas production

The clamped vial is a standard technique in anaerobic studies, even though it does not allow an easy evaluation of biogas volumes. In this study, biogas volumes were estimated by a carbon balance. By disregarding the assimilation of carbon in the microbial biomass (that is always low in anaerobic fermentations), the following carbon balance can be written

$$[\text{CO}_2] + [\text{CH}_4] = \Delta [\text{total soluble C}] \quad (1)$$

where

$$[\text{total soluble C}] = [\text{sugar C}] + 2 [\text{ethanol}] + 2 [\text{acetate}] + 4 [\text{butyrate}] \quad (2)$$

The volume of biogas at STP (0 °C, 1 atm) was evaluated by supposing ideal gas behaviour and correcting for the hydrogen fraction  $y$  in the biogas obtained by GC analysis:

$$(\text{biogas STP L})/(\text{culture L}) = 22.414 ([\text{CO}_2] + [\text{CH}_4]) / (1 - y)$$

## 3. Results and Discussion

The anaerobic consortium used in this study was sampled from a primary sludge digester. The microorganisms were first acclimated to a synthetic medium containing glucose as a sole carbon and energy source. The acclimated microorganisms were then used as inoculum for the anaerobic fermentation of *Arundo donax* hydrolysates supplemented with nitrogen and phosphorous sources plus trace elements to balance the element requirements. In contrast with previous studies (Pirozzi et al., 2012), acid hydrolysis of the cellulosic substrate was carried with HCl in place of H<sub>2</sub>SO<sub>4</sub>, since the latter treatment has been found to inhibit the growth of the anaerobic consortium.

Besides the biogas, the anaerobic fermentation products were ethanol, acetic acid and butyric acid. (Fig. 1). Together, the soluble products account for about 51% of degraded carbon. The produced biogas was 5% H<sub>2</sub>, 41% CO<sub>2</sub> and 54% CH<sub>4</sub> by volume. Biomass and biogas production rapidly slow down, probably due to inhibition by the accumulation of volatile acids. By disregarding carbon assimilation in the biomass and by assuming that all the missing carbon is in the biogas, a biogas production of 3.7 L STP/ L of culture medium was estimated from the carbon balance.

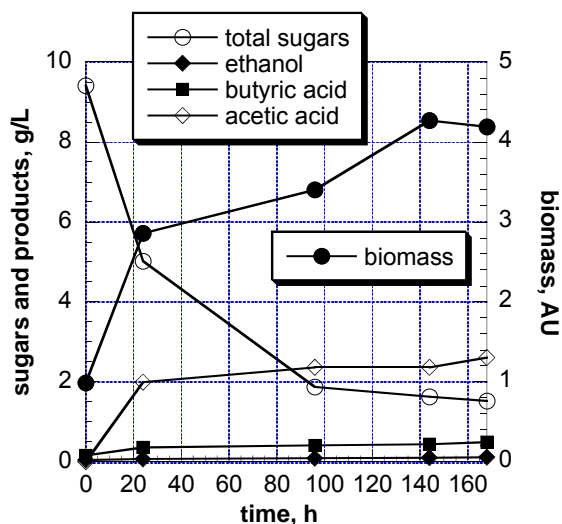


Fig. 1: Time courses of total sugars, soluble fermentation products, and biomass concentrations during *Arundo donax* hydrolysate fermentation.

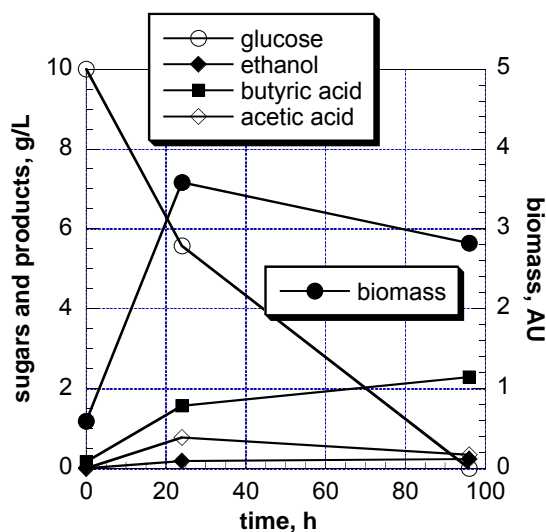


Fig. 2: Time courses of glucose, soluble fermentation products, and biomass concentrations during glucose fermentation

It is useful to compare the results with the fermentation of a synthetic medium containing glucose as a carbon source with the same inoculum (Fig. 2). In the latter case the kinetics of carbohydrate removal is faster and only about 27% of original carbon is found as soluble products. The produced biogas was 6% H<sub>2</sub>, 52% CO<sub>2</sub> and 41% CH<sub>4</sub> by volume. A biogas production of 5.1 L STP/L of culture medium was estimated from the carbon balance.

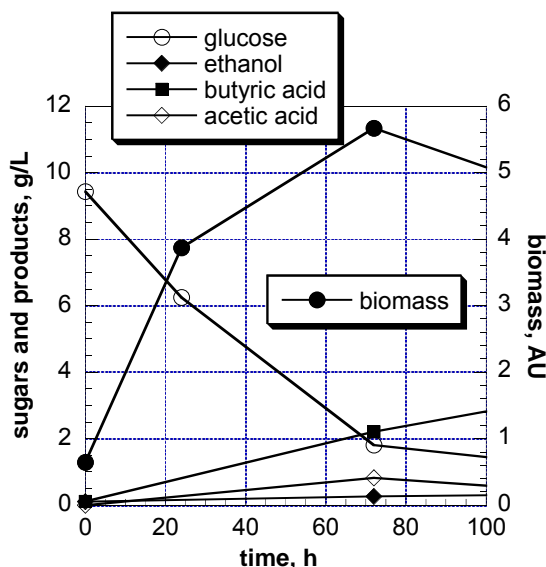


Fig. 3: Time courses of glucose, soluble fermentation products, and biomass concentrations during glucose fermentation with autoclaved inoculum.

The low hydrogen yields with both synthetic medium and *A. donax* hydrolysate are due to the persistence of methanogens in the adapted microbial consortium. As a first attempt to increase the hydrogen yield, the inoculum was autoclaved (121 °C, 15') to selectively eliminate microorganisms unable to form heat-resistant spores (as most of methanogens) in favour of spore-forming hydrogen-producing genera like *Clostridia*. Time courses of glucose, soluble fermentation products, and biomass concentrations during glucose fermentation with autoclaved inoculum are shown in Fig. 3. Disappointingly, autoclaving the inoculum was self-defeating: the kinetics of carbohydrate removal was slower than with the original inoculum and about 43 % of original carbon was found as soluble products. A biogas production of only

2.9 L STP/L of culture medium was estimated from the carbon balance without any improvement in biohydrogen yield. A possible explanation for the unexpected result could be an insufficient sterilization time with a slight reduction of methanogens and a very slow recovery of spore-forming hydrogen-producers. An optimization of autoclaving protocol is therefore necessary.

#### 4. Conclusions

Optimisation of pre-treatment of the raw lignocellulosic material is essential to obtain better yields in fermentations (Taherzadeh and Karimi 2007). Harsh conditions of hydrolysis can introduce inhibitory compounds in the substrate, as seen in the case of hydrolysis with sulphuric acid. Even with the improvement of HCl hydrolysis, kinetics remain slower and biogas yields are still inferior to those found with glucose fermentation.

Even though the yields of biogas and biohydrogen are low, the soluble products could be of interest for further processing in the biorefinery. However, further optimization of the process by imposing a selective pressure against methanogens is envisaged, in order to improve biohydrogen yields.

#### References

- Angelini, L.G., Ceccarini, L., Nassi, O., Di Nasso, N., Bonari, E., 2009, Comparison of *Arundo donax* L. and *Miscanthus x giganteus* in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance, *Biomass and Bioenergy*, 33, 635–643.
- Antonopoulou, G., Gavala, H.N., Skiadas, I.V., Angelopoulos, K., Lyberatos, G. (2008), Biofuels generation from sweet sorghum: Fermentative hydrogen production and anaerobic digestion of the remaining biomass, *Bioresource Technology*, 99, 110–119.
- Chairattananokorn, P., Penthamkeerati, P., Reungsang, A., Lo, Y.C., Lu, W.B., Chang, J.S., 2009, Production of biohydrogen from hydrolyzed bagasse with thermally preheated sludge, *Int. J. Hydr. Energy*, 34, 7612–7617.
- DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28(3), 350–356.
- Chong, M.L., Sabaratnamb, V., Shiraic, Y., Hassana, M.A., 2009, Biohydrogen production from biomass and industrial wastes by dark fermentation, *Int. J. Hydr. Energy*, 34, 3277–3287.
- Cui, M., Yuan, Z., Zhi, X., Wei, L., Shen, J., 2010, Biohydrogen production from poplar leaves pretreated by different methods using anaerobic mixed bacteria, *Int. J. Hydr. Energy*, 35, 4041–4047.
- De Vrije, T., Bakker, R.R., Budde, M.A.V., Lai, M.H., Mars, A.E., Claassen, P.A.M., 2009, Efficient hydrogen production from the lignocellulosic energy crop *Miscanthus* by the extreme thermophilic bacteria *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*, *Biotechnology for Biofuels*, 2, 12.
- Hallenbeck P.C., Abo-Hashesh M., Ghosh D., 2012, Strategies for improving biological hydrogen production, *Bioresource Technology*, 110, 1–9
- Karaoglanoglou L., Koullas D., Diamantopoulou L., Panagiotopoulos I., Papadaki S. and Koukios E., 2012, Best practices for small-scale biomass based energy applications in EU-27: the case of fermentative biohydrogen generation technology, *CET*, 29, 1117-1122.
- Kapdan, I.K., Kargi, F., 2006, Bio-hydrogen production from waste materials, *Enzyme Microbial. Technol.*, 38 (5), 569–582.
- Kotay, S.M., Das, D., 2008, Biohydrogen as a renewable energy resource – prospects and potentials, *Int. J. Hydr. Energy*, 33, 258–63.
- Lu, Y., Lai, Q., Zhang, C., Zhao, H., Maa, K., Zhao, X., Chen, H., Liu, D., Xing, X.H., 2009, Characteristics of hydrogen and methane production from cornstalks by an augmented two- or three-stage anaerobic fermentation process, *Bioresource Technology*, 100, 2889–2895.
- Mazloomi, K., Gomes, C., 2012, Hydrogen as an energy carrier: Prospects and challenges, *Renew. Sust. Energy Rev.*, 16(5), 3024–3033
- Nasirian, N., 2012, Biological hydrogen production from acid-pretreated straw by simultaneous saccharification and fermentation, *African J. Agric. Res.*, 7(6), 876–882.
- Nelson, N., 1944, A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, 153(2), 375–380.
- Pant, K.K., Gupta, R.B., 2009, Fundamentals and use of hydrogen as a fuel. In: *Hydrogen fuel: production, transport, and storage*. Editor: Gupta R.B., pp. 3–32, CRC Press Taylor & Francis Group, Boca-Raton, USA.

- Pirozzi, D., Yousuf, A., Zuccaro, G., Aruta, R., Sannino, F., 2012, Synthesis of biodiesel from hydrolyzates of *Arundo donax*, *Environ. Eng. Manag. J.* 11(10), 1797–1801
- Saratale, G.D., Chen, S.D., Lo, Y.C., Saratale, R.G., Chang, J.S., 2008, Outlook of biohydrogen production from lignocellulosic feedstock using dark fermentation – a review, *J. Sci. Ind. Res.*, 67, 962–979.
- Strobel, H., 2009, Basic laboratory culture methods for anaerobic bacteria. In Mielenz, J. R., editor, *Biofuels*, vol. 581 of *Methods in Molecular Biology*, pag. 247–261. Humana Press.
- Taherzadeh, M.J., Karimi, K., 2007, Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources*, 2, 472–499.