

HYDROGEN CONSUMPTION IN BIO-HYDROGEN PROCESS MODELLING

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Abstract

Fermentation of sugars for hydrogen production has received increasing interest as a “green fuel” process. Models describing products formation from sugar fermentation has been the focus, applied to generalize anaerobic digestion models. Such models have therefore been in continuous development. Beginning from fixed stoichiometry values as in ADM1 [1] (the most common platform in anaerobic digestion models) until variable stoichiometry with thermodynamic control [2, 3]. This article briefly describes efforts to model hydrogen production in acidogenic reactors and the most reasonable model for product formation from sugar fermentation. Present models used to simulate bio-hydrogen production fail to predict the behaviour of most such processes and it is concluded that this is because the models do not include significant hydrogen consumption mechanisms. Conceptual model modifications required to solve this problem are presented and discussed.

Key words: *Fermentation, modeling hydrogen production, hydrogen consumption, homoacetogenesis*

Nomenclature

R	= Ideal gas constant.
T	=Absolute temperature (K).
ΔG	=Change in Gibbs free energy.
ΔG°	=Standard change in Gibbs free energy.
$V_i A_i$	=Stoichiometrical coefficient i for product A_i
C_i^{vi}	=Concentration of species A_i .
q_s	=Biomass substrate uptake-rate.
q_s^{\max}	=Biomass maximal substrate uptake-rate
ΔG_{dis}	=Dissipated fraction of catabolic Gibbs free energy ΔG_{catab}
ΔG_{anab}	=Overall Gibbs energy dissipation for growth.

$\Delta G_{m/\mu}$ =Energy available for growth and maintenance purposes.

m_G =Gibbs energy dissipation rate for growth independent maintenance purposes.

$C_{AH,e}$ =Extracellular concentration of free acids.

$C_{AH,i}$ =Intracellular concentration of free acids.

X =Biomass.

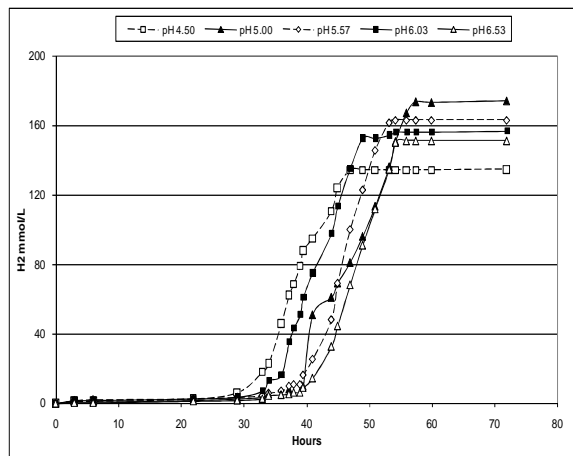
Diff_{AH} = Diffusivity coefficient.

$\Delta G_{Tr,AH}$ =Energy requirement for active transport of an acid through the membrane.

1. Introduction

Sugar fermentation using mixed cultures in batch, continuous flow stirred tank reactors (CSTR) and, lately, up-flow anaerobic sludge bed (UASB) reactors have been used to study hydrogen production potential, yields and rates. Hydrogen production in batch experiment have been successfully carried out by several investigators [4, 5], were about 50-60% of the gas produced is hydrogen. In batch experiments the observed H_2 yield correlates with the organic acid production (Figure 1). Similar results are also reported in continuous flow processes, but here the results are less consistent. CSTR experiments were therefore also carried out in our laboratory and the results consistently show that the hydrogen yield drops as the culture matures, after a strong initial production phase (Figure 2). It appears that the observed yield drops when a more concentrated biomass develops (which is required to achieve high production rates and a total conversion of the carbon source, especially for high concentration feeds). Literature data [6] support this conclusion: The hydrogen production in an ideal CSTR (with suspended, low concentration biomass) correlates with the acid production; while hydrogen yield in UASB (much more concentrated biomass) is only about 34 % of the total equivalent of organic acids. It is therefore concluded that H_2 is consumed and that the mechanisms for H_2 consumption are favoured when the biomass form dense aggregates such as biofilm and in granules typical for UASB reactors.

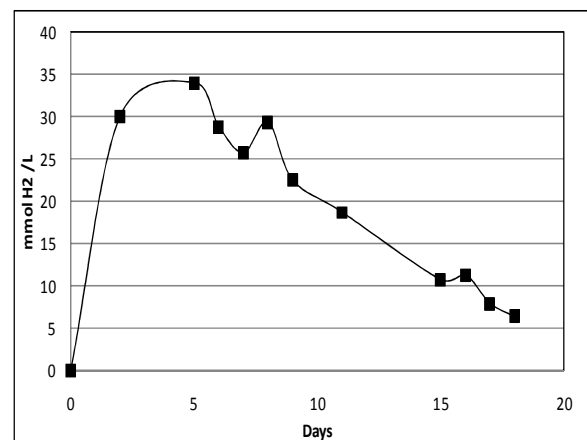
Figure 1: Hydrogen production in batch experiments, the total hydrogen production correlates with the organic acid produced.



The reactions involved in the hydrogen consumption may vary depending on several parameters, including substrate composition and pH, but it must be an issue of “electron sinks mechanisms”. We have observed a consumption rate of 2 mmol H₂/h/g-X (where g-X = gram biomass measured as volatile suspended solids) in a mixed cultured (35 °C) without methanogenic activity, where the consumed H₂ and CO₂ was supplied in head space (2:1). This is a high hydrogen consumption rate that can not be ignored when simulating such processes. Hydrogen consumption by hydrogenotrophic methanogenic and acetogenic bacteria has been extensively treated in the literature, related to syntrophic relationship in biogas processes focused on methane production [7]. Hydrogen consumption in fermentative reactors for hydrogen production has not yet reported, analyzed or modelled. Even though low hydrogen yields in bio-hydrogen reactors have frequently been observed. This is therefore the topic of our research presented here.

The pathways of reduced equivalent of glucose fermentation and/or hydrogen assimilation need to be studied more in order to determine the mechanisms of electron or proton transfer between cells in densely packed microbial aggregates. An accurate mechanistic model of bacteria consuming hydrogen or electrons equivalents in acidogenic reactors can then be developed and included in a complete process model, such as the ADM1, for process simulation. This article briefly describes efforts of modelling hydrogen production in acidogenic reactors and the most realistic model for product formation from sugar fermentation. Present models used to simulate bio-hydrogen production fail since significant hydrogen consumption mechanisms are not included. The aim of the present study is to present and evaluate conceptual model modifications required to solve this problem.

Fig 2: Hydrogen production in a CSTR (35 C), where development of a biofilm layer was observed and the production of organic acid is closely constant.



2. Modelling hydrogen production in an anaerobic chemostat reactor

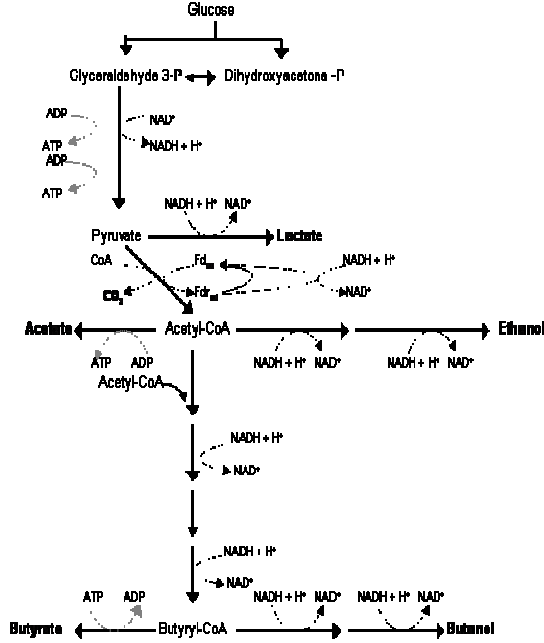
Hydrogen production in anaerobic reactors has been modelled coupled to the formation of mixed fermentation products obtained from sugar fermentation. Most of these models describe the fermentation of carbohydrates using fixed stoichiometry values [1, 8-10]. However modelling products formation by fixed stoichiometry has been unsuccessful because of the dependency of hydrogen production on several variables, such as pH, hydrogen partial pressure, organic load and kind of feed. Product formation has also been modelled using variable stoichiometry with the hydrogen partial pressure and pH as key parameters and their effects on the thermodynamic feasibility of biochemical reactions [11-13]. We consider that the last significant advance has been carried out by Rodriguez et al. [2, 3], modelling product formation from sugar fermentation with thermodynamic control. Mainly the model assumes the mixed culture as a single virtual microorganism able to carry out the most common fermentative pathways with optimum energy exploitation from the metabolic network that is proposed. Their modelling procedure is described below.

2.1 Stoichiometry: biochemical pathways

The model core is based on the theoretical description of the catabolic network (e.g. Figure 3) found in the literature [14, 15]. Substrates are converted to products by a sequence of enzymes mediated reactions. This biochemical network gives as a result a matrix of i components (substrates and products) and j processes (biochemical-chemical-physicochemical reactions, and transport processes). A component ($_{ij}$) has associated a coefficient (stoichiometrical value) so that mass balances are satisfied for each process. This

matrix can be defined in a single virtual microorganism or model as a group of microorganisms with different catabolic capabilities.

Figure 3: Biochemical network of glucose fermentation [16].



2.2 Thermodynamic boundaries and reaction control

The driving force of biochemical reactions occurring in the intracellular space depend on the Gibbs free energy change (ΔG) resulting from each reaction as follows:



$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \frac{[C]^c [D]^d}{[A]^a [B]^b} \quad (2)$$

Only a $\Delta G < 0$ makes a reaction possible. As observed from equation 2, the energy released depends on the concentration of reactants and products in the intracellular space. Modelling transport process is, therefore, fundamental in the net energy production calculation. Many anaerobic biological conversions proceed very close to thermodynamic equilibrium. Therefore the Gibbs free energy changes gives a useful way to predict if these reactions are possible or not and also to predict which are more favourable and therefore more likely to occur (note that we are not talking about rate of reactions). Still, thermodynamic laws have not been applied to generalized anaerobic models until recently by Rodriguez and collaborators [3]. The method for analyzing the thermodynamic feasibility of a specific metabolic pathway was first proposed by Mavrouniotis et al. [17] as follow:

For an individual enzymatic reaction of the stoichiometry given by

$$\sum_i v_i A_i = 0 \quad (3)$$

The Gibbs energy change is calculated as:

$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \prod_i C_i^{v_i} \quad (4)$$

The application of such “boundaries” in anaerobic models has not been properly applied, resulting in unrealistic catabolic transformation predictions against thermodynamic laws when reactions proceed with a $\Delta G > 0$, as in ADM1. Kleerebezem and Van Loosdrecht [18] discuss the application in ADM1 of a solution (eq. 5) that could be easily applied into the kinetics equations. Reactions with a $\Delta G > 0$ are just possible in syntrophic relationships as establish between acetogenic and hydrogenotrophic methanogenic bacteria.

$$if \Delta G < 0, then q_s = q_s^{\max} \cdot \{1 - \exp(\Delta G / R \cdot T)\}, else q = 0 \quad (5)$$

The same thermodynamic principle has been applied by Rodriguez et al. [2,3], not just in setting thermodynamic thresholds for a given reaction to determine if it is possible or not, but also to predict which metabolic pathway will be used by the cell under a given environmental condition. This is done using pH and hydrogen partial pressure as the main key parameters to determine the substrate conversion rate, q_s , as follow:

$$q_s = q_s^{\max} \cdot [1 - \exp(\Delta G_{dis})], if \Delta G < 0 \quad (6)$$

Here the term ΔG_{dis} refers to the dissipated energy derived from the catabolic free energy. ΔG_{dis} can then be regarded as the actual driving force for the catabolic reaction.

Lately, it has also been suggested to use Gibbs free energy for the estimation of biomass specific growth rate (μ) in anaerobic digestion models [19], demonstrated by different authors [20-22] as follow:

$$\mu = \frac{1}{-\Delta G_{anab}} \cdot (q_s \cdot \Delta G_{m/\mu} - m_G) \quad (7)$$

According to these equations (eq. 1-7), a single microorganism with a set of catabolic capabilities, can catabolise a given substrate (glucose) through the pathways that the environment, product and substrate concentration makes thermodynamically possible. The extension of the energy realised by this reactions define the kinetics by which this reaction occurs, since it is coupled to the maximal specific substrate

conversion rate q^{\max} . Energy from these reactions becomes available (as ATP) for growth. Biomass yield may, therefore, also be predicted roughly because of the relation between Gibbs energy dissipation and growth efficiency and rate. This is the most appropriate approach that has yet been applied to some extent in ADM1 for modelling products fermentation.

2.3 Transport process

Intracellular concentration of acids, protons (pH), substrates and products are important in calculating the ΔG of catabolic reactions as discussed above. Modelling transport phenomena through the membrane is also related to the generation or consumption of energy as ATP and will add to the metabolic energy production from catabolic reactions.

The concentration of protons (H^+) depends on these transport process and also the equilibrium between undissociated and dissociated form of an acid.

Two ways of transport can be identified, free diffusion and active transport. In both cases the driving force is governed by the concentration gradient between the intracellular and extracellular space. This “driving force” is accompanied by a transport resistant term as we can observe from the equations below. In active transport, production or consumption of energy is associated through the generation or consumption of proton motive force across the cell membrane. So, transport of undissociated acids against a concentration gradient will consume energy while energy is produced with a favourable concentration gradient. Energy requirement from transport (ATP_{Tr}), summed to energy need for maintenance (ATP_m) define the total energy expend for the cell (ATP hydrolysis).

The transport process and energy generation or consumption equations are detailed below (Eq.8-13).

The free diffusion of an acid into the cell:

$$V_{diff} = Diff_{AH} \cdot (C_{AH,e} - C_{AH,i}) \cdot X \quad (8)$$

Active transport of an undissociated acid molecule:

$$\Delta G_{Tr,AH} = R \cdot T \cdot \ln \frac{C_{AH,e}}{C_{AH,i}} \quad (9)$$

Formation/consumption of ATP by active transport as follow

$$ATP_{Tr} = \sum_j \frac{\Delta G_{Tr,j}}{\Delta G_{ATP}} \cdot V_{Tr,j} \quad (10)$$

Also generation of energy as ATP

$$\sum (ADP + Pi + energy) = \sum (ATP_m) \quad (11)$$

$$\sum ADP + Pi + energy = \sum (ATP_{Tr}) \quad (12)$$

$$ATP_{mTr} = ATP_m + ATP_{Tr} \quad (13)$$

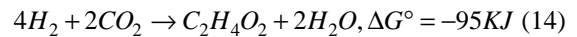
2.4 Microbial groups or a single virtual micro-organism?

The matrix of biochemical reactions used by Rodriguez et al. [2,3], assuming a single microorganisms that hold all the metabolic capabilities, makes modelling relatively simple. In reality, however, diverse microbial groups hold different metabolic capabilities. The assumption of one single virtual microorganism appear satisfactory to model steady state conditions. This approach may fail when modelling transient conditions, or long term changes that may select the most able microorganism (while the others are out competed).

Modelling fermentation based one single virtual microorganism, giving the same weight to each metabolic conversion, is also problematic when hydrogen consumption needs to be accounted for. Modelling of different bacterial groups is necessary for a complete model to also account for the homoacetogens, as discuss below.

3. Hydrogen consumption processes in acidogenic reactors.

Mixed cultures are currently use for bio-hydrogen production, mainly because they can metabolise a wide range of feed stocks, no sterilization requirements and because of adaptive capacity to varying conditions, making mixed cultures attractive for bioenergy production [23]. Acidogenic reactors with mixed cultures also, however, have the capability of metabolise the following reaction:



The presence of homoacetogens (eq. 14) should be expected in any anaerobic culture because they are the most versatile physiological group of bacteria: strictly anaerobe, fast growing and some are spore-forming organisms [24] that resist heat treatment often applied to eliminate methanogens. Homoacetogenesis was not included in ADM1 [1] because it is less relevant in traditional methane production, for which ADM1 was developed, than in hydrogen producing fermentors, for a variety of reasons, including: a) high H_2 threshold (520-950 ppm) at mesophilic conditions compared with sulphate reducers and methanogens, b) because of complex, but unknown, competition phenomena between methanogenic and homoacetogenic

organisms for H_2 and CO_2 . In absence of competition from methanogens for hydrogen, homoacetogens will proliferate, as observed repeatedly in our laboratory.

Together with molecular hydrogen consumption by homoacetogenic bacteria we suggest that the mechanisms by which electrons from acetic and butyric fermentation are “consumed” should be modelled. Decarboxylation of pyruvate to acetyl-CoA involve the transfer of 2 electrons to Fed_{oxi} to form Fed_{red} a direct electron donor for proton (H^+) reduction in the generation of molecular H_2 . However, reactions involving H_2 and $NADH_2$ generation compete for Fd_{red} thus the reduction of NAD^+ to $NADH_2$ will diminish the H_2 yield. $NADH_2$ it is the main electron carrier involved in biomass synthesis and the source of electron to reduced organic acids. Several authors have discussed that pH is the main factor coupling the oxidation of Fd_{red} with NAD or H^+ to form $NADH_2$ or H_2 [15]. This electron carriers competition is not well understood, and can, therefore, not yet be accurately predicted. This topic will be further investigated in order to be able to run realistic simulations in the future based on mathematical implementation of the proposed conceptual model.

3. 1. Modelling hydrogen consumption.

H_2 and electrons (“potential hydrogen” electrons) consumption in acidogenic reactors in the absence of methanogenic bacteria has been demonstrated experimentally in our laboratory, data not yet published [25-26]. Evidence for this is also found in data in the literature [6], but it has not yet been discussed or accounted for in the literature. Models describing directly hydrogen production are few [27]. As discussed above, the main focus has been in describing products formation from sugar fermentation (that is actually not the same). A conceptual model of how hydrogen consumption can be accounted for in a general fermentation model is shown in Figure 5. The mechanisms by which electrons from acetic and butyric fermentation are “consumed” are modelled together with molecular hydrogen consumption by homoacetogenic bacteria.

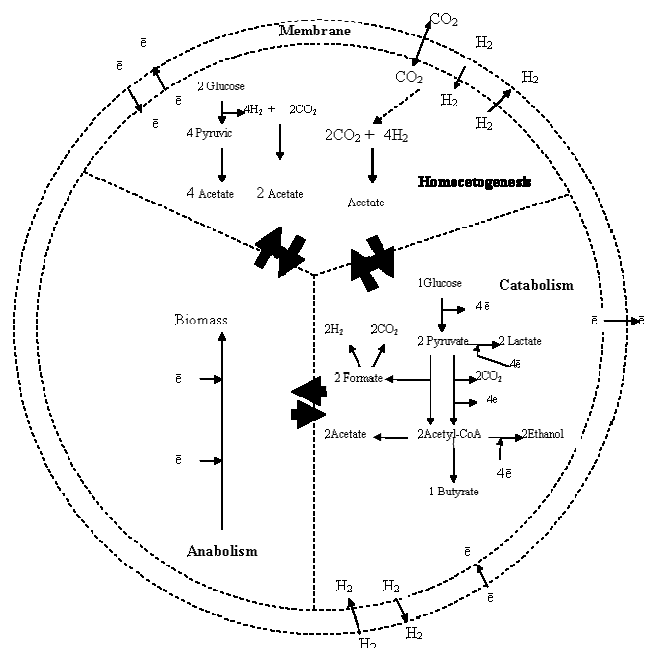
4. Conclusions

The importance of hydrogen consumption in hydrogen producing reactors is documented based on experimental observations. Hydrogen consumption is, however, not accounted for in published models presently used to simulate hydrogen production in fermentation processes. It is proposed to use a thermodynamic approach and a detailed biochemical description of the possible reactions involved to account for homoacetogenic hydrogen consumption. This approach has recently been used to simulate hydrogen production, a general model framework that can be expanded to account for consumption also. A conceptual model for this is proposed.

5. References

- [1] Batstone D.J., Keller J., Angelidaki I., Kalyuzhnyi S., Pavlostathis S.G., Rozzi A., Sanders W., Siegrist H. and Vavilin V. 2002. Anaerobic digestion Model No.1; IWA task group on modelling of anaerobic digestion processes. *IWA publishing, London, 77pp.*
- [2] Rodriguez J., Kleerebezem R., Lema J.M., Loosdrecht M.C.M. 2005. Modeling product formation in anaerobic mixed culture fermentation. *Biotechnology and bioengineering*, 93(3):592-606.
- [3] Rodriguez J., Lema J.M., Loosdrecht M.C.M. and Kleerebezem R. 2006. Variable stoichiometry with thermodynamic control in ADM1. *Water Science and Technology*, 54(4):101-110.
- [4] Lee Y. J., Miyahara T. and Noike T. 2002. Effect of pH on microbial hydrogen fermentation. *Journal Chemistry , Technology and Biotechnology*, 77:694-698.
- [5] Van Ginkel S., Sung S. and Lay J.J. 2001. Biohydrogen production as a function of pH and substrate concentration. *Environmental Science. Technology*, 35:4726-4730.
- [6] Gavala H., Skiadas I. and Ahring B. 2006. Biological hydrogen production in suspended and attached growth anaerobic reactor systems. *International Journal of Hydrogen Energy*, 31: 1164 – 1175.

Figure 4: Proposed conceptual model



- [7] Thiele J., Chartrain M., and Zeikus G. 1988. Control of Interspecies Electron Flow during Anaerobic Digestion: Role of Floc Formation in Syntrophic Methanogenesis. *Applied and Environmental Microbiology*, Jan. 1988, p. 10-19.
- [8] Kalyuzhnyi SV. 1997. Batch anaerobic digestion of glucose and its mathematical model. 2. Description, verification and application of model. *Bioresource Technology*, 59:249-258.
- [9] Vavilin VA., Rytow SV. Lokshina LY. 1996. Modeling hydrogen partial pressure change as a result of competition between the butyric and propionic groups of acidogenic bacteria. *Bioresource Technology*, 54:171-177.
- [10] Von Munch E., Keller J., Lant P., Newell R. 1999. Mathematical modelling of prefermenters. I. Model development and verification. *Water research*, 33(12):2757-2768.
- [11] Costello DJ., Greenfield PF., Lee PL., 1991. Dynamic modeling of a single-stage high rate anaerobic reactor. 1. Model derivation. *Water Research*, 25(7):847-858.
- [12] Mosey F.E. 1983. Mathematical modelling of the anaerobic digestion process-regulatory mechanisms for the formation of short chain volatile acids from glucose. *Water Science and Technology*, 15(8-9):209-232.
- [13] Ruzicka M. 1996. The effect of hydrogen on acidogenic glucose cleavage. *Water Research*, 30(10): 2447-2451.
- [14] Madigan M. and Martinko J. Brock Biology of Microorganisms. 11th edition. *Pearson Educational, London*. 992pp.
- [15] Buckel W. 1999. Anaerobic Energy Metabolism. In: Lengeler J.W., Drews G., Schlegel H.G., editors. *Biology of prokaryotes*. Oxford: Blackwell Science.
- [16] Dabrock B., Bahl H. and Gottschal G. 1991. Parameters affecting solvent production by clostridium pasteurianum. *Applied Environmental Microbiology*, 58:1233-1239.
- [17] Mavrovouniotis M.L., Stephanopoulos G., Stephanopoulos G. 1992. Synthesis of biochemical production routes. *Com. Chemistry Engineering*, 16:605-619.
- [18] Kleerebezem R. and Van Loosdrecht M.C.M. 2006. Criticizing some concepts of ADM1. 10th IWA World Congress Anaerobic Digestion, Montreal, Canada. Vol 1:199-204
- [19] Rodríguez J., Lema J. and Kleerebezem. 2008. Energy based models for environmental biotechnology. *Trends in biotechnology*, 26(7):366-373.
- [20] Heijnen JJ., Van Loosdrecht M. C. M. and Tijhuis L. 1992. A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. *Biotechnology bioengineering* 40:1139-1144.
- [21] Van Briesen JM. 2002. Evaluation of methods to predict bacterial yield using thermodynamics. *Biodegradation*, 13: 171-190.
- [22] Tijhuis L. L., Van Loosdrecht M. C. M and Heijnen J. J. 1993. A thermodynamically based correlation for maintenance Gibbs energy requirements in aerobic and anaerobic chemotropic growth. *Biotechnology and bioengineering*, 42: 509-519.
- [23] Kleerebezem R. and Van Loosdrecht M.C.M. 2007. Mixed culture biotechnology for bio-energy production. *Current opinion in Biotechnology*, 18:207-212.
- [24] Schink B. 1994. Diversity, Ecology, and Isolation of Acetogenic Bacteria. In Acetogenesis, ed. Harold L. Drake. *Chapman and Hall, London*. 197-228.
- [25] Arenillas J. 2006. Fermentative hydrogen production in anaerobic continuous flow stirred tank reactors. M.Sc. Thesis.
- [26] Gañán M. 2008. Study of hydrogen sinks in anaerobic processes for sludge treatment. M.Sc thesis. 82pp.
- [27] Chen C. C., Lin C.Y. and Chang J-S. 2001 Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. *Applied Microbiology Biotechnology*, 57:56-64