BIOHYDROGEN PRODUCTION: ONE STEP BEYOND

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Introduction. A large proportion of the world energy needs are being covered by fossil fuels, which have led to an accelerated consumption of these non-renewable resources. Lately, large efforts are being conducted worldwide in order to explore new sustainable energy sources that could substitute fossil fuels. Large amounts of biomass are available in the form of organic residues and some of these can be used after minor steps of pre-treatment, while others may require extensive chemical transformations prior to being utilized as a raw material for biological energy production. Biological processes such as methane and hydrogen production under anaerobic conditions, and ethanol fermentation are future oriented technologies that will play a major role in the exploitation of energy from biomass.

Using the appropriate microbial mechanisms of anaerobic digestion, hydrogen (biohydrogen) would be the desired product of the digestion process while the organic acids would be the by-products. The major advantage of energy from hydrogen is the absence of polluting emissions since the utilization of hydrogen, either via combustion or via fuel cells, results in pure water.

Heterotrophic fermentations for biohydrogen production are driven by a wide variety of microorganisms such as strict anaerobes, facultative anaerobes and aerobes kept under anoxic conditions. Substrates such as simple sugars, starch, cellulose, as well as diverse organic waste materials can be used for biohydrogen production. Various bioreactor types have been used and operated under batch and continuous conditions; substantial increases in hydrogen yields have been achieved through optimum design of the bioreactor and fermentation conditions.

This presentation explores the research work carried out by the IPICYT´s group in fermentative hydrogen production using organic compounds as substrates.

Results. A continuous stirred tank reactor was operated using chess whey as substrate. Three hydraulic retention times (HRT: 10, 6 and 4h) were tested attaining the highest volumetric hydrogen production rate (VHPR) at 6h. Moreover, four organic loading rates (OLR) were evaluated at a fixed HRT of 6h. The highest VHPR, 46.61 mmol H_2/l/h and hydrogen molar yield (HMY) of 2.8 mol H_2/mol lactose were attained at an OLR of 138.6 g lactose/l/d. The dominant bacterial species at HRT of 10 and 6h belonged to the *Clostridium* genus. The enhancements of both HMY and VHPR are critical to assess the full-scale practical application of fermentative processes, which are now considered as potential primary generators of sustainable energy in the near future.

Hydrogen production in high cell density bioreactors, such as UASB and packed-bed, is promising, nonetheless, unexpected methane production addressed the evaluation of operational strategies to control the methanogens. An increment of the OLR from 20 to 30 g COD/L-d increased the hydrogen production rate 172%, whereas the methane production was reduced 75%. The only strategy that completely inhibited the methane production was a second heat treatment to the biomass. However, the latter strategy also selected homoacetogens, which are hydrogen consuming organisms. The community analysis of the biomass withdrawn from UASB reactors with molecular techniques, such as PCR-DGGE and cloning, showed a proliferation of organisms related to hydrogen producers such as *Clostridium tyrobutyricum, Citrobacter freundii* and *Enterobacter aerogenes*, as well as the homoacetogens *Blautia hydrogenotrophica, Oscillibacter valericigenes* and *Clostridium ljungdahlii*. The archaeal community analysis showed that methane was produced by hydrogenotrophs from genera *Methanobrevibacter* and *Methanobacterium*.

Results of hydrogen production using oat straw and enzymatic hydrolysates as well as the microbial population dynamics in sequencing batch reactor will be presented. Perspectives and future research of the group will also be discussed.

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