Alternative energy sources are becoming more and more important, and hydrogen and methane production from biomass and residues are options for renewable energy. Currently, research is focused on optimization of hydrogen and methane production from vinasse, the liquid left in the distillation of ethanol from sugar-cane derivates. Molasse is also generated during the process of sugar and ethanol production and due its high organic content, it is also has the potential for hydrogen and methane production. Gaps related to the process, e.g. configuration, operation and stability of reactors and limited knowledge of the involved microbiota need to be overcome in order to evaluate the potential application of anaerobic technology for the treatment of vinasse and molasse and bioenergy production. In addition, the used pesticides can either remain in the environment, causing environmental problems, or transform into harmless compounds. The work proposed in this project will address the use of anaerobic technology on hydrogen and methane production from molasse. In addition, molecular tools such as qPCR assays will be developed to monitor the key players. Biodegradation tests will be performed, to identify the biodegradation potential for the used pesticides in the environment. Finally, the biodiversity in soil systems at sugar-cane production sites that have received pesticides will be studied. Both geochip and qPCR assays will be used to monitor indicator species in the contaminated soil. This is in line with the strategy that will be used for the relation between biodiversity and eutrophication in a BE-Basic project.
## Bionergy from sugarcane molasses

In order to evaluate the effect of organic loading rate (OLR), material support and source of inocula on hydrogen production, two lab-scale up-flow structured bed reactors (USBR) were operated under thermophilic condition (55°C) for 45 days at three different loading rates conditions: condition I from day 1 to day 12; condition II from 12 day to 26 day and condition III from day 26 to day 45. USBR1 was inoculated with auto-fermented vinasse and low density polyethylene cubes were used as support bed. USBR2 was inoculated with anaerobic sludge from UASB reactor treating poultry slaughterhouse wastewater and polyurethane foams were used as support bed. The bioreactors were continuously fed with sugarcane molasses.

Values of pH of influent ranged from to 5.5 to 6.5, and effluent pH values were stable during all operational period for both reactors with values from 4.0 to 4.5. The low pH values of effluents show that acidogenesis occurred. In fact acids were formed and oxidation of organic matter was incomplete with average values of COD removal of 24% (USBR1) and 30% (USBR2) for total COD and 35% (USBR1) and 37% (USBR2) for soluble COD. Hydrogen production was related with the organic loading rates, material support and source of inocula. According to the Figure 1, hydrogen production rate increased with organic loading rate but started to decrease at the end of operational period with ORL of 120 gCOD g*L⁻¹*d⁻¹. Hydrogen production was higher in USBR2 than in USBR1 starting from condition II. In USBR2 the production of acids was also higher with predominance of acetic and butyric acids, precursors of hydrogen production.

**Perspectives:** USBR2 has been in operation over 200 days to evaluate the stability of hydrogen production for long period of time. Methanogenic bioreactor was coupled to acidogenic bioreactor and the system has been in operation with crescent OLR to evaluate the bioenergy production (hydrogen and methane) by the combined system.

## Analytical method for pesticide determination in soil

Chemometric tool Box-Behnken was used as experimental design to develop a gas chromatographic method for determination of fipronil and metabolites in soil. Three variables were evaluated: (1) solvent ratio, (2) number of cycles and (3) time of ultra-sound. The variables were analyzed in pairs and according to global “desirability” (D global) (Figure 2) the best methodology of extraction of analytes from soil is use the solvents hexane:acetone in ratio of 1:1 (v/v), two cycles of ultra-sound for 15 min each.

**Perspective:** This methodology was validated and it has been used to monitor biotransformation of fipronil and metabolites in soil with historical of sugarcane plantation.

## MAIN PUBLICATIONS
