BIOTREATMENT OF LIQUID, SOLID OR GAS RESIDUES: AN INTEGRATED APPROACH

R. Auria#, P. Christen#, E. Favela, M. Gutiérrez, J.P. Guyot#, O. Monroy, S. Revah, S. Roussos#, G. Saucedo-Castañeda, and G. Viniegra-González*.

Universidad Autónoma Metropolitana, Iztapalapa, (UAMI) D.F. Mexico. CP 09340.

Institut Français de Recherche Scientifique pour le Dévélopément en Cooperation (ORSTOM) France.

Research workers from UAMI and ORSTOM have being working for the last 15 years in an effort to study solid, liquid and gas fermentation systems in order to try to understand their potential and limitations for remedial environmental purposes. Among the main results to be cited: a) A comprehensive model of mycelial growth and metabolic activity, based on experimental evidence taken at microscopic level by image analysis, gasometric studies (on line barometric and gas analysis) and thermometry (heat and mass balances). b) As a result, a new scale-up strategy for solid and gas fermentation systems has been developed based on the conservation of heat and water content of the fermentation mash. c) The limitation of biomass activities in terms of surface and volume hindrances, as a fundamental constraint for densely packed bioreactors has been studied. d) Fundamental work has been done in relation to the biochemical and microbiological mechanisms of film and particle formation in anaerobic digesters. e) New strategies have been developed for the production and selection of mutant microbial strains specially adapted to solid fermentation systems. f) New approaches have been developed for reusing spent biomass and solid residues in agriculture and livestock production.

As examples of possible applications of these studies the following can be mentioned: a) The use of *Aspergillus niger* grown on coffee pulp to reduce its caffeine level, to produce pectinases and to generate a solid residue with probiotic activity in the rumen; b) The use of bioscrubbers to remove from the air various fouling sulfur compounds in a cellophane factory; c) Removal of recalcitrant compounds in industrial waste water

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treatment by the use of advanced anaerobic reactors; d) The use of a composting process in order to produce phytostimulant compounds.

INTRODUCTION

The use of microbial biomass as a catalytic material to transform and break down chemical compounds that foul the environment is becoming one of the major alternatives to accelerate at local, regional and global scales, the rates of material recycling, in a way, which is compatible with high standards of living. One way to contribute to this worldwide effort is to study the basic models of biocatalytic activity at various levels of complexity: a) At a microscopic level, to develop and use kinetic models of microbial growth and metabolism to estimate the fundamental kinetic variables such as the observed or apparent specific growth rate (μ_{obs}), the specific metabolic rate (q_S) and the yield of biomass production. (Y). b) At an intermediate level, to develop basic relations of mass and energy exchanges and c) At a macroscopic level, to obtain the integrated results of mass and energy exchanges, together with the overall conversion processes in a given biotransformation process. Furthermore, the use of biological material makes it possible to consider the genetic plasticity of biocatalysts which can be tailored or trained to do specific biochemical jobs if there is a good command of the basic principles of dynamics, genetics and molecular biology of microbial populations.

In this paper some results related to the use of aerobic molds, anaerobic bacteria and facultative yeasts for breaking down or transforming organic residues, are presented. Special attention is paid to the integration of results obtained at different levels of research with emphasis on the use of microorganisms to transform organic residues into useful products.

A MODEL OF MICROBIAL GROWTH AND HEAT AND MASS TRANSFER FOR SOLID STATE FERMENTATION (SSF) OF ORGANIC RESIDUES.

Microscopic Model for Microbial Growth.

According to Viniegra-González *et al.*, (1993) a population of hyphae branching at an average rate ϕ will have an specific (exponential) growth rate μ_{obs} given by the following expression which is also valid for any

bacterial single cell culture where the active growth units duplicate with frequency ϕ

$$\mu_{obs} = ln(2)\phi \qquad (1)$$

Also, according to Larralde-Corona *et al.*, (1994) the value of ϕ for mycelial cultures, can be estimated by the following equation

$$\phi = u_r / L_c ln(L_c / L_o) \qquad (2)$$

being, u_r, the maximum value of the apical extension rate; L_c, the critical length after which the distal mycelial segments produce a new branch, and, L_o, the minimal length of a new segment produced by branching. Larralde-Corona *et al.* (1994) have suggested that L_c can be estimated to be nearly twice the average length (L_{av}) of the distal segments in the peripheral edge of a mycelial colony.

$$L_c \cong 2L_{av} \qquad (3)$$

which was justified by considering that L_c is the maximal value in a population of many distal segments which are distributed as a Poisson function of second order and assuming that L_c is two times the standard deviation of L_{av} then $L_c = (1 + 2/\sqrt{2})L_{av}$. Furthermore, L_o was estimated to be close to the hyphal diameter D_h.

$$L_o \approx D_h \qquad (4)$$

This microscopic model has been validated by Viniegra-González *et al.* (1993), Larralde-Corona *et al.* (1994) and Viniegra-González *et al.* (1994) by comparing the values of μ_{obs} estimated by Eqs. 1 to 4, using cultures of *Gibberella fujikuroi* and *Aspergillus niger*. This way the physiological response of a mycelial culture, measured in a fermentor (González-Blanco, *et al.*, 1993; Larralde-Corona *et al.*, 1994) or in a packed bed reactor (Viniegra-González *et al.*, 1993, 1994), can be estimated from image analysis of relatively small colonies grown on agar plates. Application of this technique has been used for the phenotypic classification of mutants and dikaryotic strains of *A. niger* which are adapted to produce pectinases in liquid (series AW99) or solid state (series AW96). The relative values $M = \mu/\mu_{max}$ of various strains of *A. niger* measured at different water

activities (a_w = 0.96 or 0.99) and in a culture medium with pectin as a sole carbon source, provide a way to distinguish fermentation phenotypes, for example, different strains that produce pectinases in different yields and adapted to different culture media (Antier *et al.* 1993a and b; Loera *et al.*, 1993). Thus the use of an adequate microscopic model of the mycelial growth is very helpful in estimating a macroscopic parameter (μ_{obs}) as a fundamental physiological response of different strains to be considered for certain processes, such as enzyme production to break down a contaminant.

Macroscopic Model of Microbial Growth.

Viniegra-González *et al.* (1993) used the microscopic model (Eqs. 1 to 4) in order to propose a new macroscopic model of mycelial growth which is reminiscent of the so called logistic equation.

$$R_X = dX/dt = \mu_{obs}[X + X_c][1 - (X/X_m)^p] \qquad (5)$$

Where, $X_c > 0$ is the critical biomass concentration at which, a synchronized mycelial culture starts branching for the first time; X_m is the maximal value of the biomass concentration and $p > 0$ is a parameter related to the strength of the self inhibitory interaction of biomass. If $X_c = 0$ and $p = 1$, Eq. 5 becomes the logistic equation. For $X_c = 0$ and $p > 0$, Eq. 5 becomes the growth equation proposed by von Bertalanffy (1957) for plant growth and used by Mulchandani *et al.* (1988) to model the kinetics of microbial cultures. For $X_c < 0$, it means that it is necessary to have a minimal (critical) concentration, X_c, for the onset of biomass autocatalytic reproduction.

Macroscopic Model of Microbial Metabolism.

Using the usual partitioning of metabolism for growth and maintenance, the rate of substrate, S, consumption R_S can be described by Eq. 6

$$R_S = -dS/dt = R_X/Y_m + mX \qquad (6)$$

where, Y_m, is the maximum biomass yield and m is the maintenance coefficient.

Heat and Mass Balance of a Microbial Packed Bed Reactor.

According to Saucedo-Castañeda *et al.* (1990) for a cylindrical packed bed bioreactor having total length, L, incubation temperature, T_b, and reactor

radius R_a, aerated with an air current having velocity u, and defining the coordinates r (radial position), and z (axial position) and, also, assuming cylindrical geometry, it is possible to define the following dimensionless variables.

$$\rho = r/R_a; \quad \vartheta = T/T_b; \qquad \varsigma = z/L; \quad \tau = t/\Theta; \qquad (7)$$

where, $\Theta = L/u$ is the characteristic time of a column aerated at velocity, u, and having length, L. The heat balance of a pseudohomogeneous packed bed reactor can be written with the help of the dimensionless numbers of Peclet (Pe) Biot (Bi) and Damköhler (Da_{III}) and the dry mass fraction, F_{dm}, and with initial value R_{si}, as follows:

$$\partial\vartheta/\partial\tau = [Ld_p/R_a^2 Pe][\partial^2\vartheta/\partial\rho^2 + (1/\rho)(\partial\vartheta/\partial\rho)] - \partial\vartheta/\partial\varsigma + \\ + F_{dm}\, Da_{III}[R_S/R_{si}] \qquad (8)$$

The dimensionless boundary and initial conditions are

$$\text{at } \rho = 1; \quad -\partial\vartheta/\partial\rho = Bi(\vartheta - 1) \qquad (9)$$

$$\text{at } \rho = 0; \quad \partial\vartheta/\partial\rho = 0 \qquad (10)$$

and

$$\text{at } \varsigma = 0, \quad \vartheta = 1 \qquad (11)$$

If the air convection is strong enough, as required to assure axial homogeneity, the term $\partial\vartheta/\partial\varsigma$ can be neglected and a simpler mathematical equation is obtained (Saucedo-Castañeda *et al.*, 1990)

$$\partial\vartheta/\partial\tau = [Ld_p/R_a^2 Pe][\partial^2\vartheta/\partial\rho^2 + (1/\rho)(\partial\vartheta/\partial\rho)] + \\ + F_{dm}\, Da_{III}[R_S/R_{si}] \qquad (12)$$

Equation 12 can be solved using computational techniques, such as orthogonal collocation or finite element integration, but for doing so it is necessary to have a functional relation between μ_{obs} and temperature which is often of the double Arrhenius type. That is, in a given lower range, the growth process is activated by heat but beyond a certain temperature, the microbial biomass is inactivated by further heating.

Otherwise, the solution of Eq. 12 would grow boundlessly (see Saucedo-Castañeda *et al.*, 1990 for more details). Calculations done by those authors were based on Eq. 12 using R_X as a logistic function ($p = 1$, $X_c = 0$) and seemed to follow up the temperature profiles at various radial positions of a 12 cm diameter cylindrical packed reactor packed with precooked cassava meal and inoculated with spores of *Aspergillus niger No. 10* .

Practical Applications

This result is important because it helps to predict the complex behavior of aerated packed bed reactors and SSF piles of different geometries and volumes. For example, it can be used to predict the outcome of anaerobiosis in the core of a composting pile of straw at different environmental temperatures or to compare different geometries, sizes and operational regimes, for solid state fermentors. An important application of such a result is the development of scale-up criteria for solid state fermentations which have been of rather empirical nature (Lonsane *et al.*, 1992). Saucedo-Castañeda (1992) has proposed that the dynamic heat balance calculated by Eq. 12 can also help to calculate water balance and in this way scale-up can be done, conserving the humidity and temperature levels in the reactor. This task can be accomplished by the use of continuous electronic input/output monitoring of aerated reactors (Saucedo-Castañeda, 1992). This is an advancement in the biochemical engineering for producing high added value compounds by the SSF technique, such as, cellulases from lignocellulosic fibers (Roussos *et al.*, 1992) pectinases from coffee pulp (Trejo-Hernández *et al.*, 1991; Boccas *et al.*, 1994; Antier *et al.*, 1993a and 1993b), blue cheese flavors from granular curds (Revah and Lebeault, 1989), odour production by growing mycelia by SSF (Christen *et al.*, 1992), yeast production by SSF for the production of ethyl acetate (Christen *et al.*, 1994), all those, as examples of SSF processes which can be used for solid waste or byproduct recovery and recycling. Also, fungal biomass can be produced from mixtures of liquid (low grade syrups) and solid (cane trash) wastes (González-Blanco *et al.*, 1990) and can be used as a source of probiotic materials (*e.g.:* bioorganic compounds enhancing rumen digestion of roughages) as suggested by Campos *et al.* (1990). Finally, rational design of composting fields is a need for the adequate disposing of municipal organic refuse and this has to be adapted to the seasonal and geographical conditions of the composting piles. In the tropics or during the summer, elevated ambient temperatures would impose different strategies for pile

design and turnover than in the cold weather because the solution of Eq. 12 is very sensitive to the values of outside temperature T_b. Thus numerical solutions of Eq, 12 could be helpful for the bioprocess engineering of solid waste handling.

PHYSIOLOGY AND GENETICS OF MOLDS FOR SSF

One of the important features of SSF is to be a heterogeneous system with spatial organization of microbial biomass, solid support, liquid nutrients and a separate gas phase. Oriol *et al.* (1988) showed that there are important water exchanges between particles of bagasse and cassava meal which have an effect on the overall efficiency of the SSF system. For example, water absorbed in bagasse particles apparently helps to increase the growth rate and amylase production of *Aspergillus niger No. 10* inoculated on cassava granules. Barrios-González *et al.* (1988) indicated that penicillin production using *Penicillium chrisogenum* was affected in a different way by the strength of solid content in the broth when the culture was submerged (shake flasks) or when it was done by SSF (packed bed columns with bagasse particles). Penicillin productivity was found to be at least one order of magnitude higher in SSF than in submerged fermentation (SmF). Trejo-Hernández *et al.* (1991) found that *Aspergillus niger CH4* cultured by SSF technique had also a much higher productivity than by the SmF technique. Solís *et al.* (1992) showed that *A. niger CH4* was stimulated to produce higher pectinase enzymes when cultured by SSF using high levels of glucose concentration (100 g/L) whereas the same strain was inhibited to produce pectinases by SmF when glucose concentration was above 10 g/L. Acuña-Argüelles *et al.* (1994) found that water activity could be depressed by adding ethylene glycol without inhibiting the growth of *A. niger CH4* very much. Those observations lead to Antier *et al.* (1993a and b) to develop a new protocol for the selection of random mutants of a wild strain of *A. niger* called *C28B25* which is deoxy glucose (DG) sensitive (DGS). The phenotype DG resistant (*dgr*) selected at high water activity ($a_W = 0.99$) was *dgr AW99* and was specially adapted for pectinase production by SmF. But if in addition to DG, ethylene glycol was also added to the selection medium in order to obtain low water activity ($a_W = 0.96$), then, a special kind of mutant was selected, called *dgr AW96* which was specially adapted to produce pectinases by SSF technique. Preliminary genetic analysis of such types of mold strains seems to support two hypothesis: a) *dgr* and

AW96 phenotypes are related to different regulatory products because reversion of *dgr* to the DGS phenotye is not accompanied by reversion to catabolic repression and also because b) Parasexual crosses (dikaryons) between two different strains (*dgr AW96-4* x *dgr AW99-iii*) revert to DGS phenotypes but enhance the *AW99* phenotype, which is quite different from the wild phenotype. Those results indicate that it is possible to produce new strains of *A. niger* which are specifically adapted to produce and excrete enzymes by SSF technique and which are genetically and physiological different to those specially adapted to SmF technique. This supports the earlier suggestion of Shankaranand *et al.* (1992) that there is "the need for an extensive screening program for the selection of a potent culture most suited to SSF system". Present work is now directed to the use of genetic engineering techniques in order to clone the genes responsible for the adaptation of *A. niger* to SSF, in order to use them as part of an expression system of enzymes with possible industrial and environmental applications.

GAS BIOTREATMENT IN PACKED BED REACTORS.

Basic Studies

Tubular reactors packed with biomass can be used as an effective way to treat gas currents either by changing the oxidation level of the inorganic compounds, *i.e.*, transforming sulphides into sulphates or by transforming organic toxic volatile organics into innocuous biological materials. In order to characterize the SSF packed bed reactors, two on-line properties ought to be measured: a) the amount of biomass present and b) the diffusion coefficients of the gases, through the porous reactor. Auria *et al.* (1990, 1992, 1993) and Auria and Revah (1994) have studied both aspects of SSF fermentation and have proposed that pressure drop, measured on-line, is approximately proportional to the biomass increase in the interstitial space between the solid support particles. They have used spherical Amberlite beads as a support, *A. niger No. 10* as a model mold culture and aerated columns as tubular reactors. In order to model this process they used Darcy's equation

$$\Delta P / L = U/K \qquad (13)$$

where, ΔP, is the pressure drop across the column of length L, aerated with an air current having velocity U and having a fluid conductivity K.

Auria *et al.* (1993) suggested that under their experimental conditions, K would change mainly due to changes in porosity ε which is, in turn proportional to the bed void fraction

$$\varepsilon = (1 - V_S)/V_t \qquad (14)$$

where, V_S , is the volume occupied by solid material and, V_t, the total reactor volume. They assumed that "the reactor bed porosity can only be due to the increase in biomass which reduces the volume occupied by the gas phase" (Auria *et al.*, 1993). Their data showed that the evolution of $\Delta P/L$ *vs.* time and biomass *vs.* time were parallel and that the relative fluid conductivity, K/K_{in}, decreased linearly with increasing biomass X (r^2 = 0.981) supporting their initial hypothesis, as indicated in Eq. 15.

$$K/K_{in} = 0.976 - 0.044X \qquad (15)$$

The extrapolated value for K = 0, found by Auria *et al.* (1993) was X_m = 22.2 mg/g support (dry basis) and agreed very well with the observed value of X_m = 21.5 mg/g support (see Eq. 5) suggesting that saturation of the interparticle space is one of the major limitations for biomass production in a packed bed reactor of the type used for SSF systems. The addition of different levels of glucose to the SSF system did not change the value of $X_m = 20.0 \pm 1.5$ mg /g of the solid support, for initial concentrations of glucose S_0 = 90, 130 and 200 g/L. Auria *et al.*, (1993) estimated a maximum packing density of 0.07. This result is important because it stresses one of the operational problems of packed bed reactors for gas biotreatment, that is, the saturation of the interparticle space by excessive biomass production. It also indicates the importance of on-line measurements of pressure drop as a basic control variable for such types of bioreactors.

Another important operational variable in packed bed bioreactors is the diffusion coefficient for gasses such as oxygen and carbon dioxide, which participate as major indications of the aerobic metabolism. Auria *et al.* (1993) have measured such coefficients in small columns packed with Amberlite, supplemented with glucose and mineral media and inoculated with *Aspergillus niger No. 10.* They found that the relative diffusivity of oxygen and carbon dioxide decreased following an inverse hyperbolic function of biomass

$$D/D_m = A/(X - X_o) \text{ for } X > X_o \qquad (16)$$

where, A and X_O are empirical coefficients. The value of X_O corresponds to a critical value below which D remains approximately constant ($D = D_m$). Equation 16 indicates that the gas diffusion constant decreases when biomass starts to occupy an excessive part of the interparticle space. Thus, biomass saturation not only hinders gas flow reducing the effective pressure head but also becomes a significant barrier for gas exchange.

Auria *et al.* (1994) have discussed their results and they consider that "the continuity of the gas phase was not broken and a low regular mesh-like growth occurred". In a recent work Auria *et al.* (1994) have observed a value of X_m = 103 mg/g support, which is higher than the value of 22 mg/g support reported earlier (Auria *et al.*, 1993). This was achieved using very high levels of glucose (So = 100, 200, 300 and 400 g/L) indicating that *Aspergillus niger* changes its maximal packing density at different levels of substrate concentration. Therefore, the use of different substrate levels, is one of the options for changing the operational conditions of a packed bed reactor due to the plasticity of microbial organisms. Perhaps it is possible to select special strains which grow very densely or very loosely packed as part of the design strategy for gas biotreatment in packed bed reactors.

Practical Applications

The idea of using continuous tubular bioreactors to treat polluted gas currents has been successfully applied in Mexico by the group of Professor Revah in collaboration with the corporate company CyDSA (Celulosa y Derivados, S.A.) which is a Mexican company based in Monterrey N.L. They developed proprietary "know how" for design and operation of bioscrubbers using microorganisms to oxidize fouling sulphides to sulphates and to remove them by a liquid-gas separation system. The technology was successfully developed and transferred from bench to pilot to semicommercial and finally to full industrial scale, and was awarded with a joint (UAMI-CyDSA) Serfin Prize of Ecology 1993.

Morales *et al.* (1994) have presented results showing the use of a bench-scale biofilter for the continuous removal of toluene from an air stream by biofiltration. They used peat as a packing material and activated sludge as the inoculum with a moisture content around 60%. After a long

adaptation period (40 days) the bioreactor reached a maximum removal rate of 25 g of toluene per m^3/h, which is a value similar to other published results (Ottengraf and van den Oever, 1983). The kinetics of toluene removal was found to be of zero order. This project has given the opportunity to put in operation a fully instrumented bench bioreactor with computerized on-line sampling and control devices and with automated gas chromatographic analysis for input-output mass balance.

FAST ANAEROBIC DIGESTION OF LIQUID WASTES.

Basic Studies

Fast anaerobic digestion (AnD) of liquid organic wastes is becoming an interesting alternative for biotreatment of many kinds of polluted effluents of municipal and industrial origin. This is especially important if the level of Biochemical Oxygen Demand (BOD) is higher than 2 g/L because for higher BOD levels AnD is more economical than aerobic digestion (AeD) due to the low solubility of oxygen in water, which in turn puts an upper limit on the mass transfer from air to the liquid phase. Unfortunately, the specific growth rate of methanogens is very low ($\mu \approx 0.01$ to $0.02\ h^{-1}$) making it necessary to develop techniques for retaining and reusing the methanogenic biomass in the reactor vessel in order to obtain acceptable hydraulic retention times for fast AnD of organic compounds. Biomass retention in fast anaerobic digesters can be achieved by using the natural tendency of anaerobic bacteria to form aggregates which become organized both from the spatial and the biochemical point of view (Trulear and Characklis, 1982; Guiot, 1991) in the form of biofilms or bioactive granules. González *et al.* (1992) have studied the evolution of metabolic activities during biofilm formation on polyethylene sheets and have provided data supporting the multilayer structure model (Guiot, 1991) suggesting the formation of at least two distinct layers within a bioactive granule; an outer layer of acidogenic bacteria and an inner core of acetoclastic bacteria. This type of study has been important in order to increase the understanding of granule formation and has helped our group to develop a new registered technology in a joint effort between UAM, ORSTOM and the National Autonomous University of Mexico (UNAM) for producing anaerobic granulated sludge starting from conventional active sludge from aerobic treatment plants. Such a technology has been licensed by UNAM to a Mexican private company (IMASA) and has been

used successfully for the start-up of a pilot and a full scale anaerobic digester (UASB type) in a brewery of the city of Toluca.

Other basic studies have been directed to the AnD of petrochemical compounds. For example, the anaerobic treatment of wastewater polluted by terephthalic acid (Noyola *et al.* 1990; Macarie *et al.*, 1992). Treatment efficiencies ranged from 44% to 74%, depending on the COD load and the reactor configuration (UASB or tubular). Best results were obtained by the tubular reactor, apparently due to its higher resistance to the toxic effects of aromatic petrochemicals (Macarie *et al.*, 1992). It was proposed to use the following treatment sequence of terephthalic acid effluents: primary settler → anaerobic digester → aerobic polishing.

Removal of inorganic pollutants from municipal and agricultural wastewaters is also a matter of great concern for improving the environmental standards of Mexico. Monroy and Sarquis (1990) have studied the use of water hyacinth (*Eichornia crassipes*) as a convenient way to remove large loads of nitrogen and phosphate. They found that BOD and nutrient (N and P) removal was inversely related to the input load to the water hyacinth pond. Analysis of their results and those reported in the literature, lead Monroy and Sarquis (1990) to propose the use of water hyacinth ponds as a polishing treatment after the secondary conventional process. It was recommended to use the pond productivity from 16 to 33 g of dry mass per day and per square meter (60 to 120 tons/Ha*year). Monroy *et al.* (1988) proposed the combined use of water hyacinth ponds and anaerobic digesters as an integrated bioprocess for heavy nutrient and heavy metal removal from wastewaters. Ilangovan *et al.* (1990) have studied the fate of heavy metals during the AnD of water hyacinth. They found that methanogenic sludge removed Pb, Cd, Zn and Cu from the digested juice obtained by grounding the leaves and petioles. Ilangovan *et al.* (1990) proposed the use of the following procedure: to remove water hyacinth from polishing ponds and separate roots from the rest because heavy metals are strongly accumulated in them; then, mill the petioles and leaves and digest the juice in a UASB reactor to remove COD as biogas and purge the sludge to remove heavy metals in the organic solid phase.

Engineering Studies.

Alvarez *et al.*, (1991) have proposed a mathematical model and a computing algorithm to simulate and control a two stage AnD system.

This model uses COD as input and CO_2, CH_4, and pH as outputs. They proposed the use of an adaptive computing algorithm using a linearized model around several pH steady states in order to control the reactor pH by means of bicarbonate addition. It was found that the model was robust but it needed gas rate measurements (CO_2 and CH_4) in order to be efficient. Recent experimental work (Monroy, unpublished results) has shown that the two stage AnD reactor should be controlled and designed independently for each one of the stages (acidogenic and methanogenic). This type of study can be scaled-up from a bench reactor to a 45 m^3 UASB pilot reactor now in operation at UAMI.

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