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


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Research workers from UAMI and ORSTOM have been 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.
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 ($\mu_{obs}$), the specific metabolic rate ($\eta_0$) 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 $\phi$ will have an specific (exponential) growth rate $\mu_{obs}$ given by the following expression which is also valid for any bacterial single cell culture where the active growth units duplicate with frequency $\phi$

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

(1)

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

$$\phi = \frac{u_r}{L_c \ln(L_c/L_0)}$$

(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, $L_0$, 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.

$$L_c \approx 2L_{av}$$

(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, $L_0$ was estimated to be close to the hyphal diameter $D_h$.

$$L_0 \approx D_h$$

(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 $\mu_{obs}$ 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
According to Zeldovich-Castagnole's model (1961) for a cylindrical packed bed reactor, the ideal and mass balance of a microbially mediated Redox reaction can be described by Eq. 6:

\[ R^* = \frac{d\ln X}{dt} = \frac{K_x}{K_x + m} \]

where, \( X^* \) is the maximum biomass yield and \( m \) is the kinetic constant of the microbially mediated Redox reaction.

Microscopic Model of Microbial Mediation

\[ \frac{d \ln X}{dt} = \frac{K_x}{K_x + m} \]

where, \( K_x \) is the affinity constant for the microbially mediated Redox reaction.

This equation is obtained from a stochastic model (1961) for the production of hydrogen gas, as defined by Eq. 6. The kinetic constant of the microbially mediated Redox reaction can be described by Eq. 6.

The kinetic constant of the microbially mediated Redox reaction can be described by Eq. 6.
Alternatively, 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), yeasts 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 chrysogenum* 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 the SmF technique. Solfs et al. (1992) showed that *A. niger CH4* was stimulated to produce higher penicillin 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
function of pressure

The function of pressure, as a basic control variable, for such types of measurements, is also required to determine the influence of the reaction conditions, as well as the pressure at which the reaction occurs. The pressure is determined by the equation:

\[ P = \frac{\text{reaction pressure}}{\text{pressure at which reaction occurs}} \]

Basic Stages

GAS BIOTREATMENT IN PACKED BED REACTORS

Environmental applications

The environmental application of gas treatment is a major concern in order to reduce the gases of odorous and hazardous substances. Gas treatment is performed by various methods, such as absorption, adsorption, or biological treatment. The most common method is biological treatment, which is capable of removing odors and hazardous substances from the gas stream. This method is used in various industries, such as the food and beverage industry, the paper and pulp industry, and the chemical industry. The gas treatment process involves the biological oxidation of the odoriferous compounds, which is achieved by the use of microorganisms that are specifically adapted to degrade the odoriferous compounds. The microorganisms are selected based on their ability to degrade the specific odoriferous compounds present in the gas stream.
\[ \frac{D}{D_m} = A \left( \frac{X}{X_0} \right) \quad \text{for} \quad X > X_0 \]  

(16)

where, \( A \) and \( X_0 \) are empirical coefficients. The value of \( X_0 \) 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 \( X_m = 103 \, \text{mg/g} \) 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 \((S_0 = 100, 200, 300 \) and \( 400 \, \text{g/L} \)) 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 m²/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
RESEARCHERS (Continued) \[UW\\(\text{MAN}\) (Kiever Publications, The)

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