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BIOREACTORS DESIGNED FOR EASE OF SCALE UP

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ABSTRACT

In the design of bioreactors and fermentors for different biochemical systems, the "scaling-down" technique was used. For a particular system, the small reactor prototype was designed considering factors and requirements for a large reactor. In effect, a design concept was done for a large reactor and then scaled down to the laboratory prototype. Therefore, problems in scale-up are minimized. New or improved designs for a biogas digester, an alcohol fermentor, and a plant cell bioreactor are presented. The bases for the design and scale-up considerations are discussed.

Introduction

For processes in biotechnology, bioreactors serve as the central point in transforming low-cost raw materials to high-valued products. Bioreactors have been used for the production of fermented foods and beverages for almost 4,000 years. The present bioreactors have attained some levels of sophistication by trial and error. Bioreactors have evolved because of the need to increase the rate of product formation and to improve the quality of the product. The aim in bioreactor design has been to minimize cost by optimizing volumetric productivity (1).

Fermentors have been designed for batch operation. Continuous and semibatch operations are preferred now for increased volumetric productivity. The catalysts can be free enzymes, cell organelles, or whole cells which could be live or dead. The catalyst can be supported by a matrix or used as free particles. Numerous bioreactors have been designed based on the combinations of the above classifications. Considering the large number of microorganisms and the differences among them, bioreactor design becomes a complicated process. Fortunately, using some physical and biochemical principles, mathematical models can be made although applications may be limited. This models can be used in scaling-up bioreactors (2).

Scaling-up and Design of Bioreactors

Scale-up deals with transferring laboratory or pilot plant data to commercial production. The fermentors, heat exchangers, evaporators, filters, and related equipment need scale-up techniques for design and proper operation.

Some methods of scale-up used are: (1) the fundamental method; (2) the semi-fundamental method; (3) dimensional analysis; (4) rule of thumb; and (5) trial and error. In the fundamental method, micro-balances are set up for momentum, heat-, and mass transfer. The resulting differential equations can be used for scale-up but the solutions are difficult except for very simple systems. In the semi-fundamental method, the equations in method (1) are simplified to yield equations that describe plug-flow, stirred bioreactors, or combinations. In dimensional analysis, dimensionless groups of parameters (that describe mechanisms) are kept constant during scale-up. Through experience, some rules that are considered to yield acceptable performance are set. Originally, trial and error was the only method available for process improvement in the absence of the other four. It is now rarely used. The design of bioreactors using the fundamental method is not possible while the semi-fundamental method and rule of thumb give only right orders of magnitude. Dimensional analysis has potential applications (2).

Kossen and Oosterhuis (2) describe three methods in the design of bioreactors. First, the design could observe the following procedure: (1) for a selected strain of microorganism, the kinetics of growth and product formation is obtained under different environmental conditions; (2) the optimal conditions for growth and product formation are chosen; (3) the kinetics are incorporated in the mass balance. The differential equation of mass-, heat-, and momentum balances results in a detailed model that relates the environmental conditions in the bioreactors with the operating variables. With this model, the operating variables necessary for optimal operation can be calculated. However, this procedure generally does not work because: (1) extensive experiments have to be done; (2) the differential equations are difficult to solve even for simple geometries; and (3) the operating variables obtained can be conflicting.

Second, in practice, rules of thumb are used. Promising strains are selected --in the laboratory using petri dishes and shake flasks wihout regard to conditions on production scale. Gradually, the strain is tried on existing bioreactors of increasing scale. The trials are continued until satisfactory production is obtained. The disadvantage of this method is that often, the small-scale experiments may have little or no use for the scale-up. However, the small-scale experiments allows for numerous screening tests.

The third way to design a bioreactor is the "scaling-down" method, which is used in designing the bioreactors described in this paper. The experiments at the small scale are performed under environmental conditions essentially the same as that physically and economically realized at production scale (3-5). Based on a limited information about the strain of microorganism or on some laboratory and small-scale results, a preliminary large-scale design is done. An educated guess of the environmental conditions expected is simulated in the small-scale. A smallscale bioreactor is operated under conditions representative of the production scale.

Bioreactors Designed

The design of three bioreactors using the scaling-down technique will be described: a biogas digester, an ethanol fermentor, and a plant cell bioreactor. These bioreactors are either novel designs or improved systems and patents are being applied for. The detailed performance characteristics of these bioreactors will be presented in separate papers elsewhere.

Rectangular Stage-wise Fixed Film Biogas Digester

The production of biogas through the anaerobic digestion of organic materials has been known as a naturally occurring phenomenon since earliest times. Simple digesters (box-type, cylindrical, spherical, etc.) of various construction materials have been used successfully for biogas production from slurries such as sewage sludge, animal manure, and plant materials, among others (6). In recent years, anaerobic digestion has been applied to organic wastewaters, such as distillery slops, food processing wastes, agricultural wastes, and industrial wastes among others. The new-generation bioreactors entrap or immobilize bacteria on the surface of support materials to produce biofilms that catalyze the anaerobic digestion (7, 8, 9). Among the newer designs are the anaerobic filter, upflow anaerobic sludge blanket (UASB), downflow fixed film, and fluidized bed. This bioreactors are also known as the high rate anaerobic reactors (10).

The design of a microbial-film bioreactor was undertaken with funding from the University of the Philippines Engineering Research and Development Foundation, Inc., (UPERDFI). The objective was to design a new microbial film bioreactor for biogas production from liquid industrial and agricultural wastes. The reactor must be able to operate continuously, must have an inexpensive but efficient microbial support system, must be able to handle a large capacity of wastes, and must be easy to scale up.

The conventional designs of biogas digesters are either cylindrical, spherical, or boxlike structures. For animal manure, water-proof concrete are commonly used. Mild steel sheets are used for cylindrical or column digesters. For a large volume digester, the use of metal sheets is quite expensive. In biogas systems where the product is not of a high value, a lower cost of capital investment is desired. Also, mild steel is susceptible to corrosion. A common design for high rate anaerobic digesters is the tower reactor. The laboratory scale reactors perform well but the scale up of the tower reactors poses structural problems.

All the possible shapes and materials of construction were considered. Finally, the design was based on a rectangular box, and the projected material of construction was water-proof concrete or ferro-cement. Since the bioreactor is to treat liquids, an inexpensive support system was also designed.

For optimum operation, a stage-wise system was incorporated. The box was divided into compartments such that the substrate passes through the bed in plugflow manner. Figure 1 shows the side view of the reactor. For each compartment, a skeletal structure holds the wires in which the support material is strung. The material is in the form of square sheets held by a stainless steel wire and provided with spacers. The support material could be waste plastic sheets, coconut shell (carbonized or un-carbonized), or any suitable waste material. Originally, a 10-liter digester was designed. Now a 100-liter digester is being fabricated, incorporating the solutions to the problems encountered in the 10-liter digester which include the problem in maintaining the liquid level in the reactor. A tilted reactor would result in dead spaces. Also, mixing is necessary in the initial stage to prevent sudden pH decrease.





Figure 2 shows the structure of the reactor (without the support system). The shape is simple, and is a space saver if the structure is built below ground level. Compared to a cylindrical tower, there would be less limitation to constructing a very large structure. The support material holder is also simple to construct although stainless steel material should be used. Nylon strings or ropes could be substituted for wires.



Figure 2. A schematic diagram of a 100-liter reactor without the microbial support.

A 100-liter bioreactor measuring 36 cm x 36 cm x 80 cm can be scaled-up geometrically to 100 cu. m. to measure 3.6 m x 3.6 m x 8.0 m (nominal). With a hydraulic retention time of five days, the large reactor can handle 20 cu. m. of liquid waste per day. A rectangular structure of reinforced concrete of the given dimension can be constructed without difficulty. The frame will measure about 3 m x 3 m x 1 m. Since the reaction is anaerobic and the flow, laminar, the operating characteristics of the small and large reactors are similar and independent of scale.

Since the design of the small reactor anticipated the conditions in the large reactor, construction would be easy and the cost at a reasonable level.

Stagewise-Stirred-Column Ethanol Fermentor

Ethanol fermentation is used for fuel and chemical feedstock production. The traditional method of production is by batch operation. Present researches aim for continuous reactor operation, increased productivity, and tolerance to inhibitors. Continuous methods result in high volumetric productivity. However, these require some form of biomass retention at high cell density. Some methods include cell recycle by filtration, membrane entrapment of cells, sedimentation or centrifugation, or gel entrapment. Some configurations used are chemostats, packed columns, or fluidized beds, among others (11-14). These methods result in the increase of volumetric productivity as shown in Table 1.

In continuous reactors, the difficulties encountered are lower substrate conversion and lower product concentration. However higher volumetric productivity can offset these disadvantages. The columnar reactor (packed bed or fluidized bed) is better than a mixed reactor due to its multistage (or plug flow) character. The

Type of bioreactor	Microorganism	Volumetric ethanol Productivity, g ethanol/L:h
Batch	yeast	2-5
Free-cell stirred reactor	yeast	6-8
Immobilized-cell stirred reactor	yeast	10-16
Hollow fiber reactor	yeast	10-30
Packed bed Immobilized cells	yeast	16-40
Tapered column with cell recycle	Z. mobilis	146

Table 1. Volumetric productivity of some bioreactors for ethanol production. (12)

packed bed reactor have channeling problems to large amount carbon dioxide production. The fluidized bed bioreactor allows better disengagement of the carbon dioxide, although in effect, the working volume is reduced considerably for high carbon dioxide production (12). Moreover, the scale-up of the fluidized bed bioreactor would pose a problem including bead abrasion. For tall columns, the hydraulic pressure could cause bead compression and high input pressure. Based on these problems the new design was conceived. The basis of the bioreactor design is the multistage distillation column, a successful chemical engineering equipment in large-scale operation. A distillation column consists of plates of stages with bubble caps that allow the transfer of vapor from a lower to a higher stage (upflow) and the transfer of the liquid from a higher to a lower plate (downflow).

Figure 3 shows the analogous design of the bioreactor system (5-stage) while Figure 4 shows one stage. Each stage in the fermentor acts like a plate of the distillation column but with a different method of material transfer. The liquid overflows from a higher stage to a lower stage through an external pipe. On the center of each stage, a pipe (open on both ends) is attached. In this manner, the broth is in the annular portion of each stage. The inner pipe acts as the duct for the removal of carbon dioxide from each stage. At the same time, the shaft is inserted from the topmost stage to the lowest stage with a mixing paddle attached for each stage. The shaft is rotated at a low speed for maintaining a uniform substrate concentration in each stage.

This bioreactor is suitable for immobilized cells in beads or for flocculating microorganisms. The problems cited above are solved, and problems in scale-up is minimized.

Plant Cell Bioreactor

The potential for the commercial utilization of plant cells for secondary metabolites has been known (14, 15). However the commercial exploitation de-



Figure 3. A five stage stagewise-stirred-column ethanol fermentor.



Figure 4. The details of one stage of the ethanol fermentor.

pends on the development of suitable bioreactor configurations. Although commercial fermentations are done in cell suspension systems, the tecnique cannot be applied to plant cells. Plant cells and tissues are shear sensitive (limits aeration), slow growing (results in problems in sterility of systems), and tend to form aggregates (nutrient transport limitations) (16). A solution to these problems is the extension of cell immobilization to plant cells. Some immobilization techniques applied are gel entrapment, covalent binding, and membrane etrapment. Gel entrapped cells (alginate and kappa-carrageenan) have been used but no large-scale units have been reported. Packed beds have problems of bed compression, high pressure drop, channeling, bead breakage and scale-up problems. In fluidized bed reactors, bead abrasion and scale-up becomes a problem. On the other hand membrane-entrapped cells have advantages such as some uniform cell environment is provided and pressure drop and fluid dynamics are independent of scale (17, 18). Shuler et al. (19) and Jose et al. (20) used hollow fiber membrane reactors for plant cell entrapment. The cells were entrapped in shell side while the nutrient medium flowed through the tube side.

The hollow fiber reactor offers some advantages such as: (1) the entrapment is gentle; (2) cell washout is prevented; (3) fresh batches of cells can be pumped in and out of the reactor; and (4) large scale hollow fiber units intended for ultrafiltration are available. Some disadvantages are: (1) special units will have to be desined for plant cells; (2) the hollow fibers are fragile; (3) plant cells have a tendency to settle which limits the full usage the surface area of the fiber bundle; (4) the present fibers are designed for ultrafiltration and could pose diffusional limitations; and (5) for short time use, hollow fibers are costly. Overall, scale up of these units will pose some problems (21).

As an alternative, Shuler *et al.* (17) entrapped plant cells between two rectangular sheets of membranes with the nutrient solution circulated on the outer sides of the two membranes (see Figure 5). On the other hand, Jose proposed the use of porous tube bioreactor for plant cells (21). As with the two previous reactor design discussed in this paper, the scaled-down technique is used.

The hollow fibers are replaced by porous ceramic or stainless tubes with pore size in the range of 15 to 50 μ m. These materials are available commercially over a wide range of sizes. The small-scale bioreactor is shown in Figure 6. Two porous tubes of different diameters are arranged in a concentric manner and assembled inside a cylindrical shell. The cells are loaded in the annulus between the porous tubes. Sterile aerated medium enters the inner tube and is forced to pass through the porous surface. The medium permeates through the cells and then passes through the outer porous tube onto the shell side where an outlet is provided.

For the large-scale bioreactor, the same configuration is used but with larger diameter tubes. Multiple concentric tubes are arranged in a parallel manner in a large shell, analogous to a shell-and-tube heat exchanger. A shell-and-tube heat exchanger is a design proven successful for large scale operation. We believe that the scale-up of this bioreactor would present minimal problems.



Figure 5. A schematic diagram of the plant cell membrane bioreactor by Shuler [17].



Figure 6. Schematic diagram of the porous tube bioreactor.

Conclusion

Because of the different natures and characteristics of microorganisms, and due to physical, chemical and biochemical constraints, the design and scale-up of bioreactors and fermentors pose problems. The method of design used for the bioreactors presented in this paper is the scaled-down technique but no clear cut steps can be followed. For the biogas reactor, the design basis was to find a practical shape that would be structurally sound and would use inexpensive construction materials including an efficient microbial support. For the ethanol fermentor, solving the carbon dioxide entrainment in the reactor was the major objective. Then the design was based on a successful proven equipment, the distillation column. The design of the plant cell bioreactor depended on the nature of plant cells and tissues. Based on the experience on the use of the hollow fiber reactors, the porous tubes bioreactor was designed.

We believe that with this method, together with modeling and simulation using computers, we will be able to design and scale-up bioreactors with ease.

References

- Cooney, C.L. 1983. Bioreactors: Design and Operation. Science. 219: 728-33.
- Koosen, N.W.F. and N.M.G. Oosterhuis 1985. Modelling and Scaling-up of Bioreactors. In Biotechnology Ed. H.J. Rhem and G. Reed. Vol. 2, VCH, Weinheim, pp. 571-605.
- Young, T.B. 1979. Fermentation scale-up: industrial experience with total environmental approach; Ann. N.Y. Acad. Sci. 326: 165-180.
- Oosterhuis, N.M.G. and N.W.F. Koosen. 1982. Scale-up of bioreactors. Proc. 2nd Eur. Conf. on Ind. Microbiol., Milan.
- Oldshue, J.Y. 1966. Fermentation mixing scale-up techniques. Biotech. Bioeng. 8: 3-24.
- Rohlich, Gerard A. et al. 1977. "Methane Generation from Human, Animal, and Agricultural Wastes". Report of an Ad Hoc Panel of the Advisory Committee on Technology Innovation. National Academy of Sciences. pp. 3-9.
- Atkinson, B.G., G.M. Black, and A. Pinches. 1980. Process intensification using cell support systems. *Process Biochem.*: 24-31 (May 1980).
- Jewell, W.J. 1980. Future trends in digester design. Proc. 1st International Symposium on Anaerobic Digestion.
- Lettinga, G., A.F.M. van Velzen, W. de Zeeuw, and S.W. Hobma. 1980. The application of anaerobic digestion to industrial pollution treatment. Proc. 1st International Symposium on Anaerobic Digestion.
- Lettinga, G., A.F.M. van Velzen, W. de Zeeuw, and S.W. Hobma. 1980. Use of upflow sludge blanket reactor concept for biological wastewater treatment, especially for Anaerobic Treatment. Biotech. Bioeng. 22: 700-3.
- Godia, F., C. Casas, and C. Sola. 1987. Process Biochem. 22: 43-48.
- Davison, B.H. and C.D. Scott. 1987. Operability and feasibility of ethanol production by immobilized Zymomonass mobilis in a fluidized-bed reactor. Ninth Symposium on Biotechnology for Fuels and Chemicals, Boulder, Colorado.
- Foust, A.S., L.A. Wenzel, C.W. Clump, L. Maus, and L.B. Andersen. 1960. Principles of Unit Operations. John Wiley and Sons. New York.
- Dougall, D.K. 1979. Production of biologicals by plant cell cultures. In Cell Substrates (J.C. Petriccinni, H.E. Hoppe, and P.J. Chapple, eds.) Plenum, New York. 1979, p. 135.
- Lee, S.L. and A.I. Scott. 1979. The industrial potentials of plant tissue culture. Dev. Ind. Microbiol. 20: 381.
- Shuler, M.L. 1981. Production of secondary metabolites from plant tissue culture-problems and prospects. Ann. N.Y. Acad. Sci. 369: 65.
- Shuler, M.L. 1986. Plant tissue culture using membranes. In Membrane Separations in Biotechnology, W.C. McGregor, ed. Marcel Dekker Inc. New York.

- Kargi, F. and M. Rosenberg. 1987. Plant cell bioreactors: present status and future trends. Biotechnol. Prog. 3: 1-8.
- Shuler, M.L., O.P. Sahai, and G.A. Hallsby, 1983. Entrapped plant cell tissue cultures. Ann. N.Y. Acad. Sci. 413: 373.
- Jose, W., H. Pedersen, and C. Chin. 1983. Immobilization of plant cells in a hollow fiber reactor. Ann. N.Y. Acad. Sci. 413: 409.
- Jose W. 1984. Ph. D. Thesis. Sucrose Hydrolysis and Phenolics Production by *Daucus carota* Entrapped in Hollow Fiber Rectors, Rutgers University.
- Jose W., H. Pedersen, and C. Chin. 1984. Sucrose hydrolysis and phenolics production by Daucus carota entrapped in hollow fiber reactors. Proceedings of the World Congress III of Chemical Engineering, Tokyo, Japan.