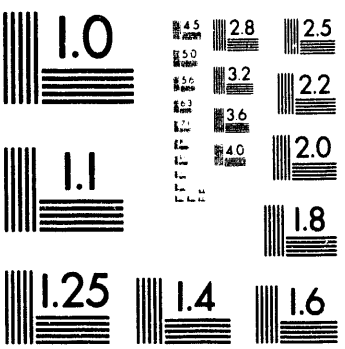

1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
7.1
8.0
9.0

1 of 1

2

THREE IMMOBILIZED-CELL COLUMNAR BIOREACTORS FOR ENHANCED PRODUCTION OF COMMODITY CHEMICALS*

B. H. Davison, C. D. Scott, and E. N. Kaufman
Chemical Technology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6226

For presentation at the
First Biomass Conference of the Americas: Energy,
Environment, Agriculture, and Industry
Burlington, Vermont
August 30-September 2, 1993

*Research sponsored by the Advanced Industrial Concepts Division — Biological and Chemical Technologies Research Program, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

875

First Biomass Conference of the Americas: Energy,
Environment, Agriculture, and Industry
August 30-September 2, 1993

THREE IMMOBILIZED-CELL COLUMNAR BIOREACTORS FOR ENHANCED PRODUCTION OF COMMODITY CHEMICALS*

B. H. Davison, C. D. Scott, and E. N. Kaufman
Oak Ridge National Laboratory
P.O. Box 2008, Oak Ridge Tennessee 37831-6226.

Abstract

Immobilized-cell fluidized-bed bioreactors (FBRs) can be used with a variety of fermentations to increase production of fuels, solvents, organic acids, and other fermentation products. Part of the increased rates and yields are due to the immobilization of the biocatalyst at high concentrations. This FBR system with immobilized *Zymomonas mobilis* increased ethanol productivity more than tenfold with 99% conversion and near stoichiometric yields. FBRs also offer several additional modes of operation for simultaneous fermentation and separation to further increase production by removing the inhibitory products directly from the continuous fermentation. The production of lactic acid by immobilized *Lactobacillus* was augmented with the addition and removal of solid adsorbent particles to the FBR. An immiscible organic extractant also was used to extract butanol from the acetone-butanol fermentation by *Clostridium acetobutylicum*. Demonstrations with these FBR systems have already shown definite advantages by improved overall product yields (decreasing feed costs) and by increased rates (decreasing capital and operating costs). Further demonstration and scale-up continue.

*Research sponsored by the Advanced Industrial Concepts Division—Biological and Chemical Technologies Research Program, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

Introduction

More than 90% of the basic feedstocks utilized for the production of non-energy products by the U.S. chemical and petroleum refining industries originate from crude oil and natural gas. Much of this material is now imported from sources that are politically unstable and that will continue to dwindle until prices become prohibitive. It is desirable to identify and develop processes for alternative renewable feedstocks, especially those that are renewable and that are environmentally benign. These processes need to include feedstock pretreatment and preparation, the conversion into the useful chemical, and the separation and purification of the desired products.

Many products can be made from renewables using conversions based on biological conversions. Bioconversions can use microorganisms or enzymes as a biocatalyst. A controlled environment (or bioreactor) is necessary to have high volumetric productivity with maximum concentration and yield of the product. Continuous operation with good process control is also desirable. At least two subcomponents need to be considered: the production of the bioreagent and the bioconversion reactor itself. Typical bioconversions occur in aqueous phase with soluble substrates and products. However, the substrate or product can be a solid, such as lignocellulose, or gas, such as oxygen. Similarly, the products can be solids, liquids, or gases. The reaction medium can be an aqueous solution, a moist gas, or even an organic liquid in contact with the biocatalytic component. An efficient biocatalyst system must be available in a bioreactor configuration that optimizes interphase contact, mass transport, and conversion kinetics.

Characteristics of an advanced bioreactor should include, if possible, high concentration of the biocatalyst, continuous operation, and excellent contact between the reacting components. Many bioreactor configurations have been proposed (such as batch, fed-batch, CSTR, and various forms of cell retention cell recycle). Ethanol production has been used with many of the bioreactors. The literature (Godia et al. 1987; Maiorella 1986) indicates that cell retention can provide substantial increases in the productivity. The conventional bioreactor system today is a large stirred tank operating in the batch mode usually with free microorganisms as the biocatalyst. Both the process itself and the downtime between batches can make the total operation take many hours or even days. A historic advantage of batch operation is the limited impact of contamination to a single batch.

Operation of bioconversion can be enhanced by utilizing continuous feed input and, thus, continuous product withdrawal as long as the feed rate is not high enough to completely wash the biocatalyst out of the system. This continuous stirred-tank reactor (CSTR) is also sometimes called a chemostat. A further enhancement is to remove the suspended biocatalyst from the reactor effluent and recycle it back to the reactor proper in order to significantly increase the biocatalyst concentration and, thus, increase the volumetric productivity of the system. This recycle can be achieved by centrifugation or, with greater success, by membrane filtration. Although there may be biological reasons that require batch operation (i.e., a sequence of required changes in the operating environment or a reaction that is intrinsically very slow), in most cases, the CSTR with cell recycle should be considered the minimum for an advanced bioreactor system. The developmental challenge for this type of reactor system is the design of large tanks in which there are good interphase contact and mixing, the establishment of optimum operating conditions and controls, and the assurance of long-term aseptic operation. Control of contamination is essential. In a CSTR, when a contaminant microbe enters the system it will compete for resources with the desired biocatalysts; under certain conditions, it will displace the desired microbe by competition.

An alternative to the conventional CSTR with cell recycle is the use of retained biocatalysts by immobilization onto integral parts of the reactor or by immobilization into or onto solid particles that will be kept in the bioreactor even at high flow rates. Two primary approaches can be used: 1) adsorption or attachment of the biocatalyst to external or internal surfaces of the solid phase; or 2) encapsulation of the biocatalyst within the particulate matrix or media. (Scott 1987) This can result in a very high concentration of the biocatalyst that does not wash out of the bioreactor. Here, the biocatalyst production step becomes a separate process for the production of large amounts of biomass or enzymes. Although the retained-cell concept can be used in stirred tanks, it is even more effective when used in columnar bioreactors. Where long residence times are required, it is best to operate as a fixed bed with larger particulates that are stationary in the reactor. For more rapid reaction, smaller particulates containing the biocatalysts can be suspended or fluidized in the column, resulting in a fluidized-bed bioreactor (FBR). This latter type of reactor may well be the best solution if it provides sufficient residence time for conversion.

Membrane-type bioreactors can also be effective retained-cell systems. In this case, the biocatalyst is immobilized on one side of the membrane with contact with the substrate maintained across the membrane. Thus, the biocatalyst environment is isolated from the reaction environment.

Many of the bioconversion steps (especially for the production of chemicals) are limited by conditions such as inhibitory product concentrations, deactivation of the biocatalyst, and dilute aqueous streams, for example. Several proposed processes seek to alleviate these types of limitations by combining several processing steps together. Two good examples of combined processes are simultaneous saccharification and fermentation (SSF) and simultaneous fermentation and separation (SFS). There are several proposed concepts for SFS where the inhibitory product is removed from the ongoing bioconversion allowing higher conversions and rates. Some of these concepts are discussed in more detail below as a technology for the production of organic acids. Simultaneous saccharification and fermentation combines the enzymatic hydrolysis of cellulose with the bioconversion step. The enzyme cellulase is inhibited by its products, glucose and cellobiose. SSF improves the rates of cellulase action by converting the sugars by fermentation into a less inhibitory product. SSF has been investigated for ethanol production. (Grohmann 1985)

Many commodity chemicals can be produced by fermentation. Research at ORNL has emphasized those systems that operate continuously with high volumetric productivity, which are most promising. Columnar bioreactors with retained biocatalysts have been particularly attractive, and three of these reactors are now described and compared with other systems.

Ethanol Production in a Fluidized-Bed Bioreactor

Immobilized *Zymomonas mobilis* was used in FBRs for high productivity and conversion production of ethanol. (Davison and Scott 1988) The bacteria were immobilized within small uniform gel beads (~ 1-mm diam) at cell loadings of up to 50 g dry wt/L. Conversion and productivity were measured under a variety of conditions, flow rates, and column sizes (up to 8 ft tall). Both laboratory media and industrial grade corn syrup and light steep water were used with identical results. (Davison and Scott 1986?) Volumetric productivities of 50 to 100 g ethanol L^{-1} h^{-1} have been achieved with residual glucose concentrations of <0.1%. The biocatalyst beads have been shown to remain active for over 2 months.

This technology has several advantages over conventional batch technology. Immobilization increases volumetric productivity by increasing cell density. The use of beads of near 1-mm diam minimizes the effect of mass transfer resistances. Fluidization allows for good interphase mass transfer and the release of large volumes of coproduct CO_2. The columnar operation allows multistage operation and localizes the high inhibitory product concentrations at the top of the reactor. This would allow a much smaller reactor with smaller capital costs to be used for the same alcohol output.

Contamination is a serious problem in the long-term operation of many continuous bioreactors. Another advantage of this FBR was the operation without asepsis. Here, nonsterile operation was successful at pH 5 due to the high flow rate and mixing, which removed the contaminant microbe while retaining the desired *Zymomonas* within the immobilized gel bead. A major advantage is the improved ethanol yield per gram dextrose of 0.49 g/g, or >97% of the theoretical stoichiometric limit, due to *Z. mobilis* compared to a yield of 0.45 to 0.47 g/g for yeast. (Davison and Scott 1988) Under current economic conditions, the raw materials (i.e., dextrose from corn or other sources) represent the largest single part of the cost; therefore, even a small but consistent increase in the yield can result in appreciable savings over the expected FBR operating lifetime of months.

Organic Acid Production and Removal

Many commercial organic acids, such as acetic, citric, lactic, and succinic acids, can be produced by fermentation. (Wise 1983) All are produced in relatively dilute form due to their high level of inhibition of the microorganism. This inhibition is due to both the chemical itself and the lowered pH from acid production. Improvements in rate have been observed using various means of cell retention including cell recycle, membranes, and immobilization. (Vickroy 1986; Ghose and Bhadra 1985) These can lead to additional problems with mass transfer, especially if oxygen is a required cosubstrate. Even with the increased rates, the final product concentration is comparable to batch reactions due to the inhibition. Conventionally, the fermentation broth is neutralized to control the pH. This yields the product in its salt form, which requires additional processing to result in the desired acid.

Several processes have been proposed to remove the inhibitory product from the ongoing fermentation. (Busche 1985) Precipitation of the organic acid salt can be done directly in the fermentation. However, the mode of retention must be considered to avoid separation problems with the precipitate. A major disadvantage of the precipitation method is the production of gypsum as a by-product. Extraction by solvents has been proposed, both direct removal of the acid and a reactive removal by forming an ester in the organic solvent phase. Configurations for extraction have included STRs and membrane reactors. Adsorption has been proposed in various forms to remove the acid from the broth. This has included direct addition into the batch STR (with problems of attrition and power); (Wang and Sobnosky 1985) passing a broth recycle stream through a side adsorbent bed; (Gaillot et al. 1990) and a direct addition and removal of the adsorbent to a fluidized bed of immobilized biocatalysts. (Davison and Thompson 1992) This last scheme is depicted in Figure 1.

The biparticle fluidized-bed fermentor is being considered for lactic acid production. Here, a continuous fluidized bed of immobilized cells would convert the sugar to lactic acid. Simultaneously, a solid adsorbent for the organic acid would be continuously added and removed from the columnar reactor. This would accomplish a dual purpose: the removal and recovery of the product and the

control of the pH in the fermentor. The essential feature of this new reactor concept is the selective removal of the adsorption particles from the FBR. This can be accomplished by exploiting a property of fluidized beds: they stratify on the basis of size or density. For example, if the adsorbent is very dense, it would migrate through the fluidized bed and be removed from the bottom of the column. Since the adsorbent itself may not have the desired physical properties, it can be immobilized as a dispersed phase within a gel bead. The size and/or density of this bead can then be controlled to give it hydrodynamic properties significantly different from those of the biocatalyst particles. For example, the adsorbent bead can be made much denser so that it could be introduced at the top of the column and removed from the base of the FBR. This would result in a countercurrent flow of the adsorbent with respect to the upward moving liquid phase.

Lactic acid is a commodity organic chemical. It is used in foods and pharmaceuticals, and there is an increasing interest in its use for the production of degradable polylactate polymers. This chemical can be produced either by fermentation or by organic syntheses. An ongoing problem has been the control of the pH in the fermentation process and the recovery of the lactate from the dilute (<5%) product stream. The pH must be controlled for the fermentation to perform effectively, and, typically, this has been accomplished by the addition of alkali with the formation of the lactate salt. The presence of other salts increases the difficulty of obtaining a high-purity product.

This biparticle FBR has been tested for simultaneous fermentation and separation of lactic acid. The bioreactor is a fluidized bed of immobilized *Lactobacillus delbreuckii.* Another solid phase of denser sorbent particles (a polyvinyl pyridine resin) was added to this fluidized bed. These sorbent particles fell through the bed, absorbed the product, and were removed. In test fermentations, the addition of the sorbent enhanced the fermentation and moderated the fall of the pH. The biparticle FBR utilizing immobilized microorganisms and adsorbent particles has been shown to enhance the production of lactic acid fourfold in this nonoptimized system. Continued work has emphasized the selection of adsorbent resins and their continuous regeneration. (Kaufman et al. 1994)

Extractive Bioconversion of Butanol

Butanol is a commodity chemical feedstock and solvent that, early in this century, was primarily made by industrial fermentation. (Jones and Woods 1986) Butanol is the primary product of the fermentation of sugars by various bacteria, in particular *Clostridium acetobutylicum.* This is a complex fermentation, with first an acidogenic phase producing butyric and acetic acids and then a solventogenic phase producing butanol, acetone, and ethanol. Both the products and the lowered pH can be inhibitory to the continued fermentation. This has limited final butanol concentrations to a maximum of 15 g/L in batch culture. The removal of the inhibitory product from the ongoing fermentation has been suggested by many researchers as a method to alleviate the product inhibition and improve the process. (Roffler et al. 1991; Groot et al. 1992)

The key advantages,compared to those of distillation, suggested for extractive bioconversion are higher feed concentrations leading to less process wastes and reduced product recovery costs. Possibilities for in situ product removal include pervaporation, (Friedl et al. 1991) the use of hollow-fiber reactors, (Shukla et al. 1989) and the use of solid adsorbents (Ennis et al. 1987), as well as the use of an immiscible extractive solvent. Key issues are the extractant toxicity and capacity as well as the actual contacting scheme devised and its operability. (Bruce and Daugulis 1991) Many solvents

have been tested for the acetone-butanol fermentation. (Shukla et al. 1989; Ishii et al. 1985; Wayman and Parekh 1987) Oleyl alcohol has been commonly used based on its low toxicity, reasonable distribution coefficient, and selectivity for butanol.

Most studies of extractive acetone-butanol fermentation have been performed in a batch reactor (Ishii et al. 1985) with free cells. Wayman and Parekh (1987) performed a sequential batch extractive fermentation with cell recycle. A fed-batch fermentation with a concentrated glucose feed continuously extracted the butanol from a recycled side stream and achieved a high butanol productivity of 1 g L^{-1} h^{-1}. (Roffler et al. 1988) A CSTR recycled the cells with a membrane filter and provided a cell-free broth to an extraction cascade. (Eckert and Shurgerl 1987) The lack of direct contact of the cells with the organic allowed the use of a more toxic extractant. An immobilized-cell FBR with a cocurrent immiscible liquid extractant (Davison and Thompson 1993) demonstrated a significant 50 to 90% increase in butanol production rate and yield in a nonoptimized extractive FBR system compared to in the nonextractive FBR. The extractant oleyl alcohol removed most of the butanol from the aqueous phase during an active fermentation in a fluidized bed with immobilized *C. acetobutylicum* for the acetone-butanol fermentation. Under continuous, steady-state operation, the butanol yield increased to 0.3 g/g with a productivity of 1.8 g L^{-1} h^{-1} when butanol was removed in this manner.

A potential extractive fermentation scheme using this process is shown in Figure 2. Here, the regenerated extractant is first contacted with the fermentor effluent to remove more of the residual butanol. It is then used to extract butanol directly in the fermentor and then regenerated to concentrate the product and close the recycle loop. Future research will emphasize use of improved strains, long-term operation, and determination of optimal conditions.

Conclusions

Immobilized-cell FBRs have been demonstrated to be a valuable class of advanced bioreactors. They provide continuous operation, high biocatalyst concentrations, and good interphase mass transfer, thus resulting in higher productivity and often improved product yields. The improved yields may be due to the cell retention by immobilization, which allows less substrate to go to the production of more biocatalyst and thus more to the product itself. This has been shown in three separate configurations and microbial systems, two including in situ product removal. Further configurations can be envisioned, and further effort is still needed to scale and commercialize these proposed designs.

Acknowledgements

Research sponsored by the Advanced Industrial Concepts Division — Biological and Chemical Technologies Research Program, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

References

Bruce, L. J., and A. J. Daugulis. 1991. *Biotechnol. Prog.* 7:116-124.

Busche, R. M. 1985. Chap. 9 in **Biotechnology: Applications and Research.** Lancaster, PA: Technomic Publishing, Inc.

Davison, B. H., and C. D. Scott. 1986. *Biotechnol. Bioeng. Symp.* **17**:629-632.

Davison, B. H., and C. D. Scott. 1988. *Appl. Biochem. Biotechnol. Symp.*, **18**:19-34.

Davison, B. H., and J. E. Thompson. 1992. *Appl. Biochem. Biotechnol.* **34/35**:431-439.

Davison, B. H., and J. E. Thompson. 1993. *Appl. Biochem. Biotechnol.* (in press).

Eckert, G., and K. Schugerl. 1987. *Appl. Microbiol Biotechnol.* **27**:221-228.

Ennis, B M, N. Qureshi, and I. S. Maddox. 1987. *Enzyme Microb. Technol.* **9**:672-675.

Friedl, A., N. Qureshi, and I. S. Maddox. 1991. *Biotechnol. Bioengr.* **38**:518-527.

Gaillot, F. P., C. Gleason, J. J. Wilson, and J. Zwarick. 1990. *Biotechnol. Prog.* **6**:370-375.

Ghose, T. K., and A. Bhadra. 1985. p. 701-727 in: Moo-Young, M., (ed.), **Comprehensive Biotechnology**, vol. 3. New York: Pergamon.

Godia, F., C. Casas, and C. Sola. 1987. *Process Biochem.* **22**:43-48.

Grohmann, K., R. Torget, and M. Himmel. 1985. *Biotechnol. Bioeng. Symp.* **15**:59-80.

Groot, W. J., R. G. J. M. van der Lans, and K.Ch.A.M. Luyben. 1992. *Process Biochem.* **27**:61-75.

Ishii, S., T. Masahito, and T. Kobayashi. 1985. *J. Chem. Eng. Japan* **18**:125-130.

Jones, D. T. and D. R. Woods. 1986. *Microbiol. Rev.* **50**:484-524.

Kaufman, E. N., S. P. Cooper, and B. H. Davison. 1994. *Appl. Biochem. Biotechnol.* (in press).

Maiorella, B. L. 1986. p. 861-914 in: Moo-Young, M., (ed.), **Comprehensive Biotechnology**, vol. 3. New York: Pergamon.

Roffler, S. R., H. W. Blanch, and C. R. Wilke. 1988. *Biotechnol. Bioengr.* **31**:135-143.

Roffler, S. R., T. W. Randolph, D. A. Miller, H. W. Blanch, and J. M. Prausnitz. 1991. Chap 6 p 133-172 in **Extractive Bioconversions**, B. Mattiasson and O. Holst, eds., New York: Marcel Dekker, Inc.

Scott, C. D. 1987. *Enzyme Microb. Technol.* **9**:66-73.

Shukla, R., W. Kang, and K. K. Sirkar. 1989. *Biotechnol. Bioengr.* **34**:1158-1166.

Vickroy, T. B. 1986. p. 761-776 in: Moo-Young, M., (ed.), **Comprehensive Biotechnology**, vol. **3**. New York: Pergamon.

Wang, H. Y., and K. Sobnosky. 1985. *ACS Symp. Ser.* **271**:123-131.

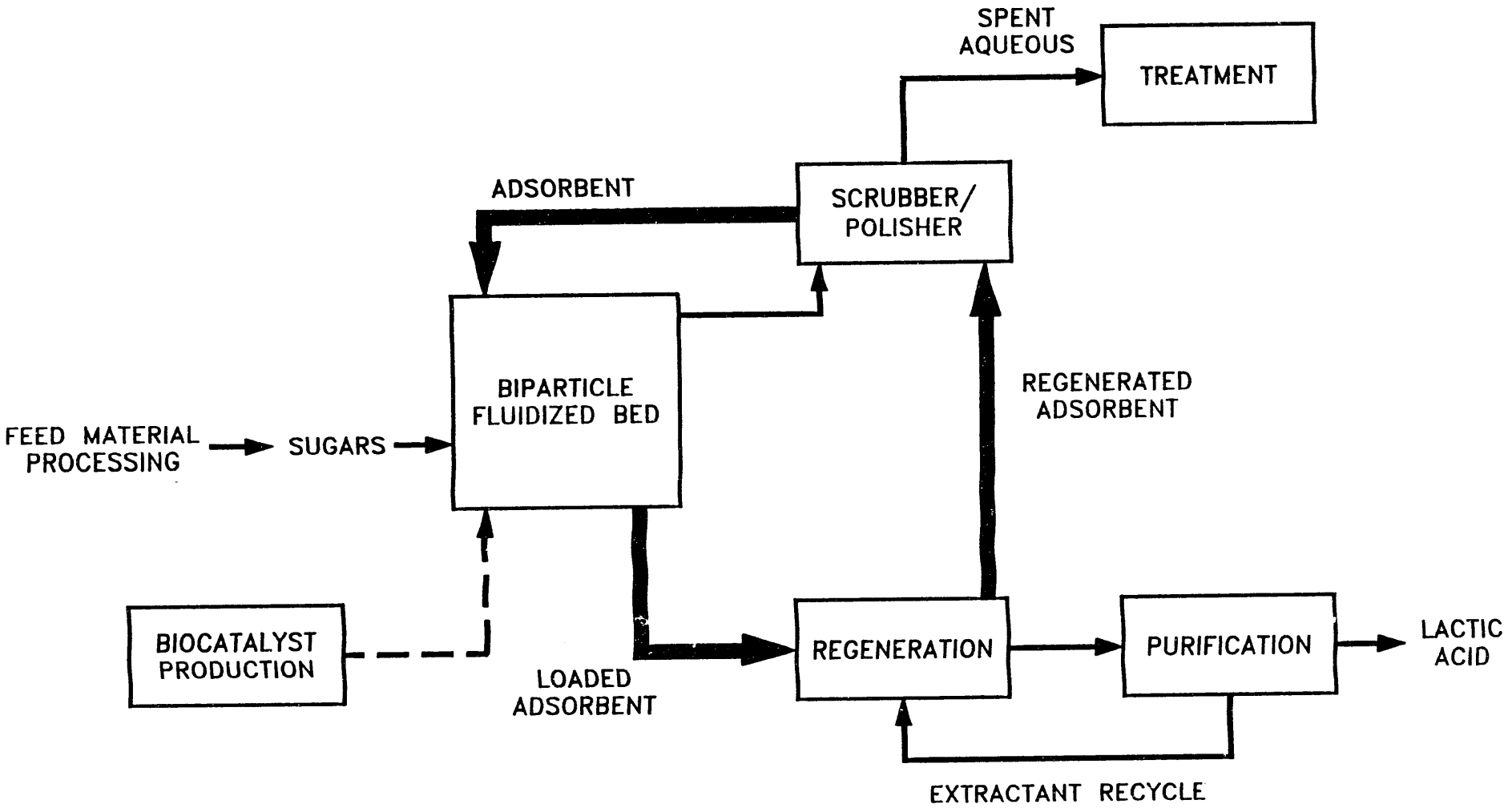
Wayman, M., and R. Parekh. 1987. *J. Ferment. Technol.* **65**:295-300.

Wise, D. L., ed. 1983. **Organic Chemicals From Biomass**, Benjamin/Cummings Publ. Co.

Figures

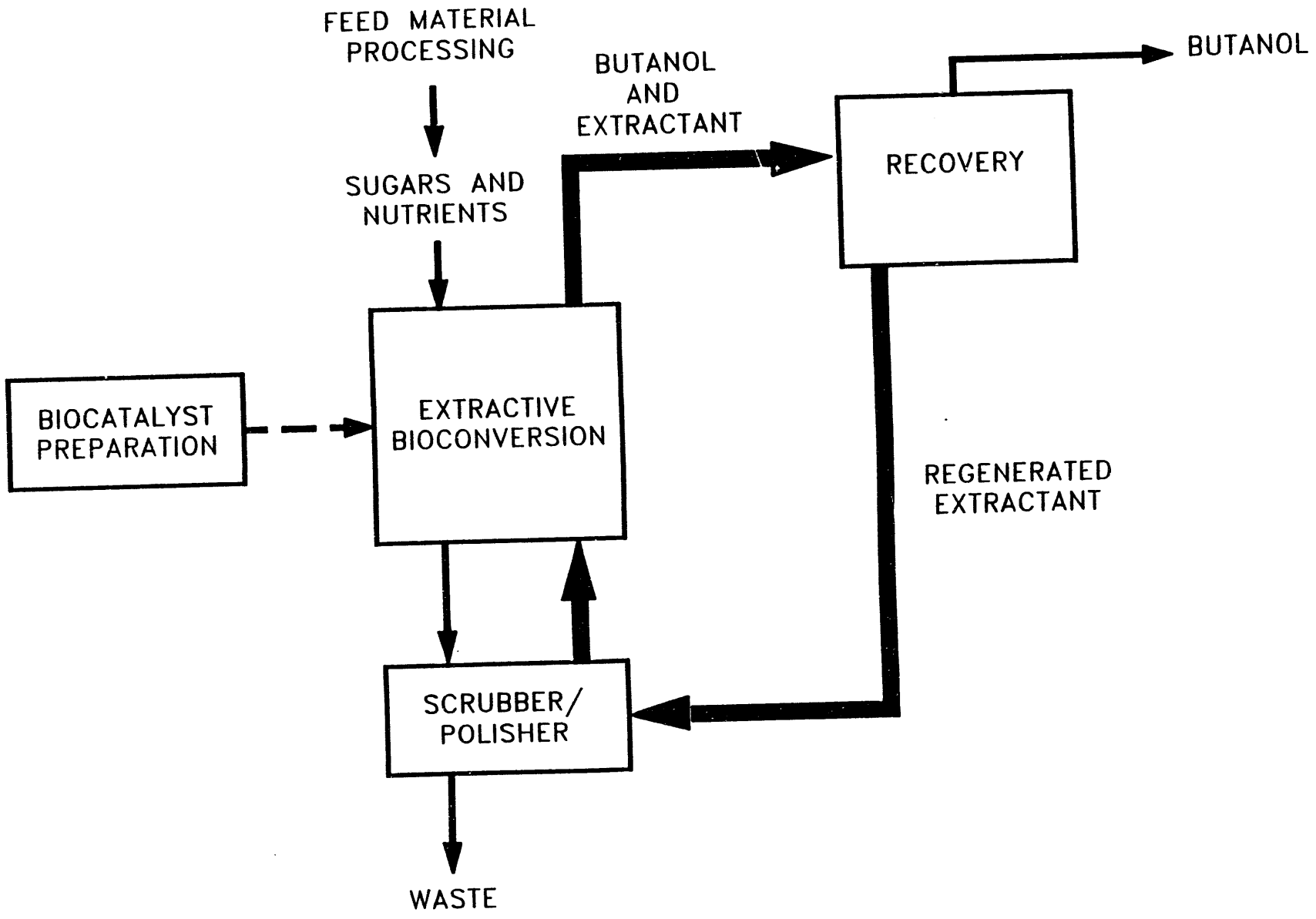
ORNL DWG 92A-444

POSSIBLE ADVANCED PROCESS SCHEMATIC FOR LACTIC ACID



ORNL DWG 92-00615

POSSIBLE ADVANCED PROCESS FLOWSHEET FOR BUTANOL PRODUCTION



DATE
FILMED
9/29/93
END