DOE/CE/40772--T5

The Production of Fuels and Chemicals from Food Processing Wastes & Cellulosics

Final Research Report
for the U.S. Department of Energy, Industrial Waste Program,
Office of Industrial Technologies: Agreement # DE-FG02-85-CE40772

Purdue University
June 15, 1997

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Industrial Co-Sponsors: Bio-Process Innovation Inc, Permeate Refining Inc., Merrill Iron & Steel, Lamont Sign Co., Xylan/Xymax Aquionics Inc., Kenyon Enterprises, Dairy Farm Products Co., Wisconsin Dairies Inc.

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Table of Contents

Appendices

*Appendix 2.1	50 Liter Immobilized Cell Reactor Separator (ICRS)
*Appendix 2.3	7,500 L ICRS
*Appendix 2.5	24,000L Continuous Stirred Reactor/Separator
*Appendix 3.1a	Lactic acid production
*Appendix 3.1b	SSF of Cellulose to Lactic Acid
*Appendix 3.2	Diacetyl Production w/ Lactic Acid
*Appendix 3.3a	Anti-fungal compounds from Cont. Plant Cell Culture
*Appendix 3.4a	Yeast from Low Levels of Lactic acid
*Appendix 3.4b	Yeast from Multiple Low Level Substrate
*Appendix 3.5a	Gluconic Acid from Waste Glucose streams
*Appendix 3.6b	The Xylan Pretreatment Process for Cellulosics
*Appendix 3.6c	Xylose Fermentation to Ethanol
*Appendix 3.7	Immobilized Cell Reactor Studies
*Appendix 3.7a	Molasses/ Sucrose to Ethanol w/ the ICRS
*Appendix 3.7b	Thermophilic Conversion of Lactose with Immob. Cells
*Appendix 3.7c	Acetone Butanol Ethanol fermentation in an ICRS
*Appendix 3.8	Flocc. yeast for High Cell Density Fermentations
*Appendix 3.9	Osmolality model for Inhibition of Ethanolic Fermentations
*Appendix 3.10	Fermentation of Permeate Mother Liquor to Ethanol
*Appendix 3.11	SSF of Raw Starch to Ethanol in a CSRS

* = Reprints — Removed for separate processing

1.Project Summary/Abstract

1.1 Project Objectives

High strength food wastes of about 15-20 billion pounds solids are produced annually by US food producers. Low strength food wastes of 5-10 billion pounds/yr. are produced. Estimates of the various components of these waste streams are shown in Table 1. Waste paper/ lignocellulosic crops could produce 2 to 5 billion gallons of ethanol per year or other valuable chemicals.

Table 1. US Supplies of Waste Substrates for Conversion to Higher Value Products

Waste stream	Approx. Quantity (kg db x 10^9)
whey/ whey permeate	1.36
potato wastes	0.32
molasses	2.4
citrus wastes	2.2
misc. food processing	2.5
yard wastes	17
municipal paper waste	60
straw/corn stalks	730
paper pulp wastes	200
sugar beet wastes	5.4
sugar cane bagasse	12.5

Current oil imports cost the US about $60 billion dollars/yr. in out-going balance of trade costs. Many organic chemicals that are currently derived from petroleum can be produced through fermentation processes. Petroleum based processes have been preferred over biotechnology processes because they were typically cheaper, easier, and more efficient. The technologies developed during the course of this project are designed to allow fermentation based chemicals and fuels to compete favorably with petroleum based chemicals. Our goals in this project have been to:

- develop continuous fermentation processes as compared to batch operations
- combine separation of the product with the fermentation, thus accomplishing the twin goals of achieving a purified product from a fermentation broth and speeding the conversion of substrate to product in the fermentation broth.
- utilize food or cellulosic waste streams which pose a current cost or disposal problem as compared to high cost grains or sugar substrates.
- develop low energy recovery methods for fermentation products, and finally
- demonstrate successful lab scale technologies on a pilot/production scale and try to commercialize the processes.

The scale of the wastes shown in Table 1 force consideration of 'bulk commodity' type products if a high fraction of the wastes are to be utilized. One such commodity is ethanol. Fuel ethanol has the capacity to absorb up to 150 billion pounds of fermentable feedstocks based on 10% ethanol usage in gasoline in the US. Ethanol production has the further advantages of reducing the net production of CO_2. If biomass boilers are used, there is little net CO_2 production when ethanol is burned. Ethanol also has the beneficial effects of reducing air pollution, reducing dependence on foreign oil, improves US balance of trade, reduces grain surpluses, and providing jobs. A second fermentation based commodity which could soak up considerable feedstock is lactic acid, if a consumer demand for bio-degradable poly-lactic plastic is developed.

The basic goal of this project has been to develop process improvements in the fermentation of waste streams to useful fuels and chemicals, and reduce the energy costs for product purification. Fermentation products examined in our project included ethanol, acetone/butanol, lactic acid, single cell protein (yeast) from both dilute and concentrated substrate streams, gluconic acid, acetic acid, co-production of flavor compound diacetyl with lactic acid, and the production of high value anti-fungal compounds and anti-cancer taxanes via plant cell tissue culture.

1.2. Summary of Project Accomplishments, Publications, Theses

Over the course of this project, a variety of processes and separations have been examined. This work, on the bench, pilot and production scale has led to several processes that are currently in commercial application, and we are hopeful that much of our other published work will form a basis for other commercially useful processes. During this project, 7 students completed pH efforts, 3 students completed MSE thesis projects, and over 14 undergraduate students completed research internships on various projects. Some of these students were British engineering undergraduates from Bradford University (4 different students), France (3 students) and Purdue students in the Ag and Biol. Food Engineering majors and Chemical Engineering Dept. (7 students). Thus this project has had far reaching educational benefits to a multitude of students. A brief chronological overview of our project's accomplishments follows:

1987:

- Granting of patent # 4665027 on ICRS concept based on Dale's Ph.D. work .
- Development of spiral wound scaleable immobilize cell fermentation matrix, (BPI/ Yang, Dale, Okos)
- Begin development of models of various unit operations for economics and design of concentration/ separations/ heating of food and fermentation products (Moyer, Okos)
- Begin work on demonstration of the application of the ICRS concept to the Acetone-Butanol- Ethanol fermentation (Park, Okos, & Wankat)
- Begin work on determination of nutritional needs of immobilized yeast (Chen, Dale, Okos)
- Begin work on screening for thermophilic *Z. mobilis* strains for industrial alcohol (Lin, Dale, Okos)
- Complete work on describing liquid residence time distributions for adsorbent trickle flow type reactors (Patterson, Dale, Okos).

Presentations

Havlik, S., P. Moyer, and M.R. Okos. Computer-Aided Food Process Design. ASAE, December, 1987.

Publications

Novel Fermentation Process Converts Whey Permeate to Fuels, Chemicals. The Cheese Reporter. October 2, 1987.

Thesis:

Patterson, J.P. Liquid Residence Time Distributions in an Absorbant Matrix for a ICRS. MSE Thesis, Agri. Eng. Dept. Purdue University.

1988:

- Lab scale demonstration of use of immobilized Z. mobilis for high rate ethanol production from glucose, rates of up to 150 g/L h reported (Lin, Dale & Okos, 1988)
- Determination of nutritional requirements for immobilized cells (Chen, Dale and Okos)
- Development of a model of for performance of a population of adsorbed immobilized cells as a function of temperature, ethanol which predicts steady state live cell fraction as a function of growth and death rates (Dale, Chen).
- Modeling, and economic comparison of 4 different methods for ethanol recovery from dilute vapors such as the product stream from the ICRS (Koo, Dale, Okos, 1988)
- Development of the food processing simulation program and introduction of fermentation, heat exchange, and recycle modules to this package (Havlik, Dale & Okos)
- Preliminary work began on the application of the ICRS concept to extreme thermophiles (Lineback & Okos)
- Complete mass transfer and liquid RTD studies on a scalable ICRS matrix designed to 1) immobilize via adsorption a high density of cells, 2) provide good gas liquid contact for gas stripping of volatile fermentation products, and 3) provide near 'plug flow' type flow of both gas and liquid phases in a counter current contactor (Dale, Guntrip)
- Design and construction of a 50 liter 6" OD stainless steel pilot ICRS system for testing of various substrates, packing methods, start-up / shut down procedures, and performance specifications (J. Smith & Dale).
- Design, construction and installation of a 1000 liter pilot scale ICRS system with a feed absorber column, partial condenser, and hot hold feed system. The system was installed at a Ft. Wayne dairy and designed to run on acid whey permeate. The design feed rate to the system was 7 to 12 liters/min (Dale)

Presentations

Dale, M.C., Y. Koo, S. Havlik and M.R. Okos. 1988. "Comparison of Recovery Processes for Ethanol from an Immobilized Cell Reactor-Separator." AIChE, Denver, CO, Aug.

Lin, J., M.C. Dale, M.R. Okos. 1988. "Environmental Effects on Ethanol Production by Immobilized Z. mobilis. " SIM, Chicago, IL, Aug.

Dale, M.C. and M.R. Okos. 1988. "Scale Up and Performance of an ICRS for the Production of Ethanol from Whey Lactose." SIM, Chicago, IL, August.

Lin, J.J., M.C. Dale and M.R. Okos. 1988. "Improvement of Bioreactor Performance: Ethanol Production by Zymomonas mobilis in an Immobilized Cell Reactor-Separator." IFT,St. Louis, MO, June.

Park, C.H., M.R. Okos, and P.C. Wankat. Effects of pH and Temperature on the Ethanol Fermentation by Free and Immobilized Yeast. AIChE, August, 1988.

Park, C.H., M.R. Okos, and P.C. Wankat. Butanol (ABE) Fermentation with Product Separation in an Immobilized Cell Reactor-Separator (ICRS) (Mathematical Modeling and Analysis). AIChE, August, 1988.

Park, C.H., M. Okos, and P. Wankat. Continuous Production of Butanol-Acetone in an Immobilized Cell Trickle bed Reactor. AIChE, November, 1988.

Theses

Chen, C. 1988. Environmental Effects on Growth and Ethanol Fermentation of Immobilized *Kluyveromyces fragilis*. pH Thesis, Food Science Dept

Lin, J.J. 1988. Environmental Effects on Ethanol Production by Immobilized *Zymomonas mobilis* in a Trickle-Flow Fermenter. pH Thesis, Purdue University, Food Science Dept

1989

- Continuous fermentation of lactose to ethanol and acids by extreme thermophiles was demonstrated on a small scale. Lactose concentrations above 2% were found to reduce the ethanol yield (Lineback & Okos).
- Extended operation of immobilized *A. acetobutylicum* in plug flow type and ICRS type reactors was demonstrated. Some degeneration of the performance was noted (Park, Okos, & Wankat)
- Long term performance of immobilized *Z. mobilis* type bacteria was demonstrated. High temperatures reduced growth and clogging tendencies of the organism. (Lin, Dale & Okos)
- The 50 liter ICRS was completed and operated for periods of up to 2 weeks continuous operation (Dale, Smith & Okos), and then was operated successfully with no contamination problems and near complete lactose utilization for a period of over 60 days on concentrated acid whey permeate from Dairy Farm Products Co. in Orville, OH (Dale, Moelhman).
- The 1000 Liter ICRS pilot plant was operated on a semi-continuous basis over 1989. A hot hold feed system was implemented to try to sterilize the feed to the system. Contamination of the reactor by bacteria proved to be a problem, with difficulties in maintaining sterile conditions in the ICRS (due to leaks) and the feed (due to high initial bacterial count) resulting in less than complete lactose utilization in the reactor. (Guy, Dale & Okos)

Presentations

Dale, M.C., S. Havlik and M.R. Okos.1989. "The Production of Ethanol from Whey Permeate using an Immobilized Cell Reactor-Separator." Wisconsin Center for Dairy Research. Madison, WI, March.

Dale, M.C. and M.R. Okos. 1989. "Design and Performance of an Immobilized Cell Reactor and Separator for the Production of Ethanol and Other Solvents." 5th Intl. Congress on Engineering and Food. Cologne, Germany, May.

Havlik, S.E., M.R. Okos, and G.V. Reklaitis. Design and Simulation Methodology for Food Processing Modeling. Presented at the 5th Intl. Congress on Engineering and Food. Cologne, Germany, May 28 - June 3, 1989.

Lee, W.S. and M.R. Okos. Production of Flavor Compounds from Cheese Whey. Presented at the 1989 AIChE Meeting, Philadelphia, PA, August 1989.

Havlik, S., A. Balint, and M. Okos. Food Operations Oriented Design System: A Steady State Food Process Design and Analysis Program. Presented at the

1989 ASAE Winter Meeting, New Orleans, LA, December 1989.

Publications

Dale, M.C., M. Moelhman, and M. Okos. 1989 "Whey Lactose Conversion to Ethanol in a 50 Liter Pilot Plant Immobilized Cell Reactor-Separator." Research report to Dairy Farm Products Inc. Orville, OH

Park, C.H., M.R. Okos, and P.C. Wankat. 1989. Characterization of an Immobilized Cell, Trickle Bed Reactor during Long Term Butanol (ABE) Fermentation. Submitted to Biotechnology and Bioengineering.

Park, C., M.R. Okos, and P.C. Wankat. 1989. Acetone-Butanol-Ethanol (ABE) Fermentation in an Immobilized Cell Trickle Bed Reactor. Biotechnology and Bioengineering, 34:18-29.

Park, C.H., M.R. Okos, and P.C. Wankat. 1989. Ethanol Fermentation using Immobilized and Free Saccharomyces cerevisiae: Effect of pH and Temperature. Submitted to Enzyme and Microbiology Technology.

Theses

Park, Chang Ho. 1989. Simultaneous Fermentation and Separation in an Immobilized Cell Trickle Bed Reactor: Acetone-Butanol Ethanol (ABE) and Ethanol Fermentation. Ph.D. Thesis, Agricultural Engineering Department, Purdue University.

Havlik, Steven E. 1989. Computer Aided Design and Modeling of Food and Food By Product Processes with Case Studies in Whey Processing alternatives. MS Thesis, Agricultural Engineering Department, Purdue University.

Lineback, D.S. 1989. Thermophilic Ethanol Production from Lactose. M.S. Thesis, Food Science Department, Purdue University.

1990

- Nutritional requirements for immobilized *K. fragilis* studies were completed. These studies indicated that high rates of fermentation could be maintained with minimal nutritional inputs to the media. The amount of free amino acids in whey permeate are thus found to be adequate for complete fermentation of the lactose. (Chen, Dale & Okos)

- The effect of various environmental factors (osmolality, temperature, and nutritional salts) on immobilized *Z. mobilis* were studied. Conditions were identified which allowed long term operation without cell overgrowth/ clogging of the reactor. (Lin, Dale & Okos).

- The design of a parallel plate adsorbed cell reactor matrix for ICRS type reactors was modeled and optimized. The effects of matrix thickness and gap spacing were design parameters, with reaction rates, mass transfer, and cost of operation determined as functions of these basic packing sizing parameters. (Dale & Guntrip).

- The interacting effects of pH and temperature on *S. cerevisae* were studied for batch operations. Higher temperatures and lower pH (40 C, under pH 4.0) prevented growth and fermentation. (Park & Okos).

- The extension of the ICRS type concept to stirred reactors (Continuous Stirred Reactor- Separator or CSRS) is conceived & modeled. (BPI: Dale)

- Studies on the use of the ICRS for extreme thermophiles continued. An organism (*T. ethanolicus*) was identified as being the best performer of several tested, and an ICR was operated for several months. Operation of the unit as an ICRS would require very careful design to keep oxygen from killing the organisms. (Lineback & Okos).

- The possibility of producing higher value flavor compounds was investigated. A number of compounds were identified in terms of market volume and market price. Based on these studies, production of diacetyl was chosen for further investigation. (Lee & Okos)

Presentations

Dale, M.C., A. Eagger, B. Truax and M. Okos. 1990. "Osmolality Effects on K. fragilis Growth and Productivity in Batch and Immobilized Cell Reactors" AICHE paper 286b, Chicago, IL. November

Dale, M.C, J. Smith, and M. Okos. 1990. "Whey Lactose Conversion to Ethanol in a 50 Liter ICRS." AICHE paper 286f, Chicago, IL. November

Dale, M.C, D. Guntrip, and M. Okos. 1990. "Design, Performance, and

Optimization of a Structured Packing for Simultaneous Reaction and Separation." ACS paper 350, Washington DC, August.

Dale, M.C. 1990. "Commercial Applications of Membrane Filtration Systems in the Dairy Industry." Conference for Advanced Technologies in Dairy Processing. Richmond, IN. March.

Lee, W. and M.R. Okos. Production of Diacetyl by Continuous Fermentation and Simultaneous Product Separation. Presented at the AIChE 1990 Annual Meeting, November 15, 1990.

Publications

Chen, C., M.C. Dale and M.R. Okos. 1990."The Long Term Effects of Ethanol on Immobilized Cell
Reactor Performance." Biotechnology and Bioengineering. 36:975

Chen, C., M.C. Dale and M.R. Okos. 1990. "Minimal Nutritional Requirements for Immobilized Yeast." Biotechnology and Bioengineering. 36:993

Dale, M.C., C. Chen and M.R. Okos. 1990. "The Effects of Ethanol and Temperature on Immobilized Cell Reactors: Cell Growth and Death Rates as Critical Factors in Reactor Modeling and Design." Biotechnology and Bioengineering. 36:983

Lin, J.J., M.C. Dale and M.R. Okos. 1990. "Ethanol Production by Zymomonas mobilis in an Immobilized Cell Reactor Separator." Journal of Process Biochemistry. 25:61

Park, C-H., M.R. Okos, and P.C. Wankat. 1990. Acetone-Butanol-Ethanol (ABE) Fermentation and Simultaneous Separation in a Trickle Bed Reactor. Accepted for publication in Biotechnology Progress.

1991

- Fermentation of highly concentrated sucrose and molasses streams to ethanol in small ICRS columns was demonstrated. Up to 50% sucrose could be fermented using osmophilic strains of yeast (Perrin & Dale)

- Preliminary work on simultaneous fermentation and separation of lactic acid was begun. This work focused on extractive fermentation due to the non-volatile nature of lactic acid (Venkatesh, Okos & Wankat).

- Work on the aerobic conversion of low level lactic acid to yeast bio-mass is begun with the intent of using the lactic acid component remaining in bottoms water following ethanolic fermentation of acid whey. (Dale, Truax, Moelhman & Salicetti)

- The simultaneous production and separation of a volatile flavor compound, diacetyl, was demonstrated. The effects of oxygen tension, pH and lactic acid were studied. Diacetyl as a secondary metabolite during lactic acid fermentation was chosen for study, and high level production of the secondary metabolite was shown (Lee & Okos)

- The preliminary design of an ICRS for acid whey permeate concentrate for Dairy Farm Products was initiated based on the successful pilot scale tests. A 60" diameter ICRS (8,500 L) design was completed. (Dale & Stwalley)

- An ICRS for sweet whey permeate concentrate was designed for Swiss Valley Farms Inc. A 55" diameter ICRS was specified. (Dale & Stwalley)

Presentations

Dale, M.C., L. Salicetti-Piazzo, B. Truax, and M. Okos. 1991. "Conversion of Dilute Lactic Acid to Single Cell Protein." ASAE Paper #916585, Chicago, IL, December

Venkatesh, K.V., M.R. Okos and P. Wankat. Production of Lactic Acid in Membrane Recycle Bioreactor Utilizing lactose. AIChE meeting, November, 1991, Los Angeles, CA.

Publications

Lin, J., M.C. Dale, and M. Okos. 1991. "Osmotic (a_w) Effects on Growth and Ethanol Production of Free and Immobilized Z. mobilis." Process Biochem. 26:143.

Thesis

Lee, W. S. 1991. Diacetyl Production using Free and Immobilized Cells. Ph.D. Thesis, Agricultural Engineering Department, Purdue University.

1992

- Lab scale work on the conversion of multiple low level substrates in fermentation waste waters to yeast begins (Dale, Salicetti, Okos, Wankat)
- Lab scale conversion of cellulose to lactic acid is completed. Work on extractive fermentation of lactose to fermentation is continued. (Venkatesh, Okos & Wankat).
- Diacetyl production in a continuous stirred sparged reactor as a secondary fermentation product during a lactic acid fermentation is demonstrated. (Lee & Okos)
- Lab scale work begins on the production of high levels of yeast from concentrated whey permeate substrates (BPI/Kenyon Enterprises, Dale)
- Begin evaluation of using ICR techniques for production of plant cell tissue on a continuous basis (Choi, Okos)
- Design & modeling work begins on evaluation of various methods for ethanol dehydration, and the concept of integrating solvent absorption and extractive distillation (SAED) with gas stripping of ethanol in ICRS and CSRS type reactors is begun. (BPI/ Dale)
- Design of a unified absorber/ICRS with internal ducting is completed. Begin fabrication of unified stainless steel 7,500 L. ICRS by Merrill I&S (Dale, Harris, Hinner).
- Begin work on development and scale-up of spacerless packing for ICRS with industrial partners (Dale, Kenyon Enterprises, Merrill Iron & Steel, Permeate Refining Inc)
- A patent on the CSRS concept spin-off of the ICRS is granted. (BPI/ Dale

Presentations

Dale, M.C. 1992. "Design and Construction of a 7,500 Liter Immobilized Cell Reactor Separator for Ethanol Production from Whey Permeate". AICHE paper 161a Miami. November.

Salicetti, L., M.C. Dale, M. Moelhman, M. Okos, and P. Wankat. 1992. "Free and Immobilized Yeast for BOD Reduction in Dairy Waste Streams". AICHE Annual Meeting, Paper 165k, Miami. November.

Dale, M.C. 1992. "Production of Ethanol, Lactic Acid or SCP from Biomass". Biobased Products Expo '92. St. Louis. October.

Lee, W.S., M. Okos, and M. Dale. 1992. "Production of a Butter Flavor

Compound
Diacetyl) with Simultaneous Product Separation". AICHE paper 8d. Minneapolis, MN. August.

Dale, M.C. and M. Moelhman. 1992. "A Low-Energy Low-Capital Process for Corn to Ethanol" Corn Utilization Conf. St. Louis. June

Dale, M.C, D. Guntrip, M. Moelhman, and M. Okos. 1992. "Production of Ethanol in an Immobilized Cell Reactor-Separator." China-USA Bio-Reaction & Bioseparation Conference, Hangzhou, P.R.C., June

Dale, M.C., 1992. "Design and Analysis of Continuous Bio-reactors Utilizing Immobilized Living Cells". China-USA Bio-Reaction & Bioseparation Conference, Hangzhou, P.R.C., June

Dale, M.C., 1992. "Food and Non-Food Uses for Whey". Ohio Dairy Producers Conference, Columbus, OH, March.

Venkatesh, K. V., Okos, M. R., Wankat, P. C., Modeling of Simultaneous Saccharification and Fermentation of cellulose to lactic acid. AIChE summer meeting, Minneapolis, August 1992.

Dale, M.C. 1992. Production of Ethanol, Lactic Acid or SCP from Biomass. Biobased Products Expo 92. St. Louis. October

Publications

Venkatesh, K. V., Okos, M. R., Wankat, P. C., 1992. Kinetic model of growth and lactic acid production from Lactose by Lactobacillus bulgaricus. Accepted for publication in Process Biochemistry, (in print).

1993

- Lab scale experiments on simultaneous saccharification and fermentation of raw starch to ethanol are started (Michel, Dale)
- An bench scale aluminum 4 liter rectangular CSRS is built and tested for fermentation of sucrose, lactose, and raw starch (Lei & Dale)
- Strains of flocculating yeasts are compared for high density yeast fermentations in tower and CSRS type reactors (Dale, Zhao)
- An Immobilized Cell, 200 Liter High Density Aerobic Yeast Reactor (AYR) was built and performance tested with 1 month operation in Wisc. and 2 month operation @ Purdue (Kenyon Enterprises/ Harris, Dale & Moelhman)
- Lab scale and modeling efforts on aerobic BOD reduction from dairy and brewery waste water using yeast production are continued (Salicetti, Okos, Wankat, Dale & Moelhman)
- Preliminary experiments on production of gossypol (an anti-fungal) compound by immobilized plant cells begin (Choi, Okos, Heinstein)
- The 50 L ICRS is modified to test for pilot scale tests of SAED low-energy ethanol recovery process (Dale & Moelhman)
- The construction of the unified 7,500 Liter ICRS was completed, and the unit was installed at Permeate Refining Inc. in Hopkinton, IA

Presentations

Dale, M.C. 1993. A Continuous Low-Energy Process for Ethanol from Corn, Starch and Biomass. Governors' Biomass Ethanol Coalition. Peoria, IL. November.

Dale, M.C. 1993. "A Low Effluent Process for Ethanol Production from Biomass", CPBR Symposium, November, Chicago.

Dale, M.C. 1993. "The Solvent Absorption/Extractive Distillation (SAED) Process for Ethanol Recovery from Gas Streams" AICHE COFE meetings, February, Chicago

Dale, M.C., S. Michel, and M. Okos. 1993. "A No-Cook Process for the conversion of Starch to Sugars for Ethanol Production" AICHE COFE meetings, February, Chicago, IL

Salicetti, L., M. Okos & P.C. Wankat. 1993. Modeling of Lactose Assimilation by the Yeast, K. marxianus. Conf. on Environment, Purdue Univ., August.

Publications

Dale, M. Clark. 1993. Ethanol Production from Whey in an Immobilized Cell Reactor. AICHE Chpt One 7:2 22-28

Venkatesh, K.V., M.R. Okos and P.C. Wankat. 1993 Immobilized Cell Reactor (ICR) to Produce Lactic Acid from Lactose. Submitted to Biotechnology Progress.

Venkatesh, K.V., M.R. Okos and P.C. Wankat. 1993. Continuous Production of Lactic Acid by Bioprocess with Cell Recycle and Hollow Fiber Extractor. Submitted to Biotechnology Progress.

Venkatesh, K.V., M.R. Okos and P.C. Wankat. 1993 A Hollow Fiber, Immobilized Cell Reactor Separator (ICRS) to Produce Lactic Acid by Extractive Fermentation. Submitted to Biotechnology Progress.

Thesis

Venkatesh, K.V. 1993. Lactic Acid Production using Extractive Fermentation. Ph.D. Thesis, Chemical Engineering Department, Purdue University.

1994

- A Solvent Absorption/Extractive Distillation System for 7,500 Liter ICRS is conceived and preliminary design calculations completed. (Dale)
- The use of highly flocculent yeast strains is studied for use in cascade and CSRS type fermentations (Dale, Zhao)
- The 50 Liter pilot scale ICRS is modified to test the SAED system for ethanol recovery from vapor streams. (Dale, Moelhman)
- Research demonstrates ability to continuously generate and release plant cell metabolite, gossypol, from an immobilized plant cell reactor (Choi, Okos, Heinstein)
- Dual culture Aerobic Yeast Reactors (AYR's) are built and operated to demonstrate BOD reduction of 'bottoms water' with yeast reactor on bench scale (Dale, Moelhman)
- Begin work on pretreatment and fermentation of cellulosics (Xylan/ Dale, Zhao)
- Develop and model growth of multiple strains of yeast on multiple (low level) substrates (Salicetti, Okos & Wankat)
- The 7,500 L ICRS is tested, modified, and operated @ the PRI site using concentrated whey permeate (Dale, Lehman)
- Design, and begin construction of 24,000 Liter CSRS for fermentation of starch/cellulosics (BPI/Lamont /PRI/ Dale.)

Presentations

Dale, M.C. 1994. "Reduction of High BOD Distillery Wastes via Yeast Production in a Gas Continuous Reactor" AICHE paper #23b, San Francisco.

Dale, M.C., S. Lei and C. Zhao. 1994. "No-cook Conversion of Starch to ethanol in a 4 L. and 24,000 L. CSRS. AICHE paper #23h, San Francisco.

Publications

M.C. Dale, A. Eagger, and M.R. Okos. 1994. "Osmolality Effects on Free and Immobilized K. fragilis Growth and Productivity in Whey Permeate Concentrate." Process Biochemistry. 29:535-544

Dale, M.C., N. Perrin, and M. Okos. 1994. "Production of Ethanol from Concentrated Sucrose and Molasses Solution Using S. Pombe in an Immob. Cell Reactor Separator." AICHE Symp Ser #300 Vol 90:56-62.

1995

- Begin evaluation of applying plant cell reactor techniques to taxane (anti-cancer agent) (Choi, Okos, Heinstein, Dale)
- Complete work on evaluation of thermo-mechanical pretreatment of cellulose to allow enzymatic release of sugars (Xylan/ Dale, Lei)
- Bench scale development of a flocculent Pichia stipitus yeast for xylose fermentation (Dale, Zhao)
- Modeling of Solvent Absorption Extractive Distillation process using Aspen Plus is completed. (Zhao, Dale)
- The application of the CSRS to biomass in a dual yeast fermentation system is conceived and lab development is started (BPI, Indiana Biomass Program/ Dale)
- The CSRS is operated on a semicontinous basis for over six months as a cascade reactor system using flocculent yeast (PRI, BPI, DOE- ERIP/ Dale, Lehman)
- Begin construction of 32,000 Liter Aerobic Yeast Reactor (AYR) at PRI site for waste water BOD reduction (Iowa Ec. Dev, Permeate Refining Inc, BPI/ Dale, Lehman)

Presentations

Dale, M.C. 1995. "A Pilot Scale Reactor/Separator for Ethanol Production" AICHE CoFE Chicago, Nov.

Dale, M.C. 1995. "An Overview of Dairy Processing By-Products and Wastes: Current and Future Utilization and Research Needs" Invited Speaker. AICHE CoFE Chicago, Nov.

Dale, M.C, S. Lei and C. Zhao. 1995 "A Lab and Pilot Scaled Continuous Reactor Separator for the Production of Ethanol from Sugars, Starch or Biomass Streams. Biomass of Americas Conf. Portland, August.

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Theses

Choi, H.J. Elicitation and Permeabilization of Immobilized Plant Cells for the Continuous Production of Gossypol. Ph.D. Thesis, Ag. Engineering, Purdue University

1996

- Continue work on taxane production in an immobilized plant cell reactor (BPI, NIH/ Choi, Dale, Moelhman, Okos, Heinstein)
- Begin development of steep biomass pretreatment for enzymatic biomass conversion to ethanol (BPI/ Dale, Moelhman)
- Continue continuous operation of 24,000 L CSRS system on continuous basis as cascade reactor at PRI site (PRI/ Dale, Lehman)
- Install 2 column SAED system for ethanol recovery and dehydration from 24,000 L CSRS system @ PRI (PRI/ Dale, Lehman)
- Complete construction of 55 L six stage CSRS for pilot scale dual culture conversion of cellulosics to ethanol(Indiana Biomass, BPI/ Dale, Boeger, Lehman)
- Begin development of biomass to pulp/ ethanol / lignins process (BPI/ Dale, Moelhman)

Presentations

Dale, M.C., D. Gibb, and R. Lehman. 1996. Performance of a 24,000 L. Continuous CSRS for Ethanol form Sugars and Application of the CSRS towards Cellulosics. Bio-Energy '96 Conf. Nashville TN, Sept.

Choi, H., M.C. Dale, P. Heinstein, and M. Okos. 1996. Continuous Taxol Production using an Immobilized Plant Cell Reactor. ACS New Orleans, March.

Publications

Dale, M.C., M. Moelhman, and C. Zhao. 1996. Design of a Pilot Scale CSRS with Solvent Absorption and Extractive Distillation. AICHE Symp. Series. In Press.

Dale, M.C. 1996. Dairy By-Products: Current Utilization and Research Needs. AICHE Symp. Series. In Press.

Thesis

Salicetti, L. 1996. Modeling of Multiple Species of Yeast Grown on Multiple substrates. Ph.D. thesis, Chemical Engineering Dept.

1997

- Development of uses for solublilized lignin (Indiana Biomass, BPI/ Dale, Moelhman)

- Development -demonstration of lab scale continuous beer process (BPI/ Dale, Moelhman)

- Development of productive media for plant cell reactor taxane reactor (BPI/ Purdue/ Okos)

- Development of osmoltolerant K. fragilis yeast strain for the fermentation of whey permeate mother liquor (MCF, BPI/ Dale, Moelhman)

- Test and modify pilot scale SAED system at PRI site (PRI/ Dale, Lehman)

- Installation and operation of industrial scale (2X 96,000 Liters) Cascade Stirred Reactor for the fermentation of permeate mother liquor (MCF/BPI/ Dale, Moelhman, Walker)

Presentations

Dale, M.C., M. Moelhman, and S. Walker, 1997. Industrial Scale Production of Ethanol from Whey Permeate Mother Liquor. ASAE, Minneapolis. August

Publications

Dale, M.C., 1997. A Low Energy Continuous Reactor/Separator for the Production of Ethanol from Starch, Whey Lactose, Molasses or Cellulosics. ERIP Final Report DE-FG01-94CE 15594

Dale, M.C. and M. Moelhman, 1997. Development of a High Efficiency Ethanol from Cellulosics Process and Evaluation of Uses for By-Products. Indiana Biomass Final Report

Dale, M.C., M.R. Okos etc. 1997. The Production of Fuels and Chemicals from Food Processing Wastes and Cellulosics. DOE Final Project Report.

1.3. Projected Industrial Implementation of Processes, and Projected Energy and Waste Savings

Industrial Implementation

Permeate Refining has been operating the 24,000 CSRS on a continuous basis for over 2 years operation. Feed rates are held at between 2 and 4 GPM. Total fermentation of 20% waste candy rinse and starch totals approximately 5 million pounds of wastes converted to ethanol by this reactor alone.

Permeate Refining also introduced a 160,000 liter continuous cascade fermentation system designed by Dale based on experience gained with the CSRS. This system operates at between 15 and 20 GPM, and has been running continuously since June, 1996, with only one or two short shut downs. Total wastes converted to ethanol by this system averages 35,000 #/day, or 12 million pounds per year.

Minnesota Clean Fuels Co. has built and implemented two, 3 stage 96,000 liter cascade fermentation systems to convert waste permeate mother liquor and starches to ethanol. This system was based on designs by Dale using the osmolality model for inhibition of growth and productivity. The two systems have a design operation rate of 10-12 GPM each for a waste sugar usage rate of over 40,000 #/day or 15 million pounds/yr.

We are also hoping to interest a number of whey producers and other food processing waste generators in the CSRS/SAED technologies. These groups will be invited to the PRI open house, which should be scheduled in the near future.

Developing Spin-off Technologies

Several spin-off technologies are being developed based on fundamental studies completed during this project.

1) Taxane (anti-cancer compounds) in immobilized plant cell reactors.
2) Continuous beer.
3) Cellulose conversion to ethanol/pulp/ lignin chemicals using the CSRS

Estimated Annual Energy Savings in Year 2010

Energy savings will accrue from:

1. The CSRS for ethanol production from food wastes: If we assume that 40% of the high sugar/starch food wastes produced in 2010 are converted to ethanol with the total fermentable food processing waste volume estimated at 2.85×10^{10} #/yr., 0.88×10^{9} gallons of ethanol could be produced.
   a) Fuel energy created @ 84,000 BTU/gal -----> 7.4×10^{13} BTU/yr.
   b) Energy saved over conventional ethanol tech. -----> 1.9×10^{13} BTU/yr.
   c) Energy saved from not treating sugars as waste @ 1KW-h/1 # BOD -----> 4.0×10^{13} BTU/yr.

2. Utilization of the CSRS lower energy technology for conversion of corn/starch to ethanol: if 20% of ethanol producers switch or upgrade from current energy usage of 40- 60,000 BTU/gal to the Purdue technology with an estimated energy usage of 28,500 BTU/gal., and 50% of the new add-on ethanol capacity expected by the year 2010 (Current ethanol production is out 0.95 billion gal/yr., while Year 2010 is conservatively estimated at 1.96 billion gal/yr. (Eth. Alert, Neb. Energy Office, Aug, 1992), so .19 billion gal due to upgrade, .51 billion gal due to new capacity using CRS technology leads to 0.7e9 gal. ethanol from corn/starch in year 2010.
   a) Fuel energy created @ 84,000 BTU/gal -----> 5.8×10^{13} BTU/yr.
   b) Energy savings over conventional technology -----> 1.5×10^{13} BTU/yr.

3. Utilization of the CSRS lower energy technology for the conversion of waste paper to ethanol: Assuming 20% of waste paper is used as a feed stock at .35# ethanol/# paper, and 1.5e11# paper per year is produced (Opportunities for Energy form MSW, Goodman and Walter, 1990) 1.6×10^{9} gallons of ethanol will be produced.
   a) Fuel energy crated @ 84 MBTU/gal -----> 1.3×10^{14} BTU/yr.
   b) Energy savings over conv. tech. -----> 3.52×10^{13} BTU/yr.
   c) Landfill volume reduced @ 15#/ft3 -----> 2×10^{9} ft3/yr

4. Utilization of biomass crops/municipal yard wastes for ethanol w/CRS technology Assuming 18 billion #/yr. are used in ethanol production with .3# ethanol per pound biomass -----> 0.5 x 10 9 billion gal/yr.
   a) Fuel energy created @ 84 MBTU/gal -----> 4.2×10^{13} BTU/yr.
   b) Energy savings over conv. tech. -----> 1.1×10^{13} BTU/yr.
   c) landfill space for 50% which are MYW @ 5#/ft3 -----> 1.8×10^{9} ft3

5. Utilization of our low energy technology for yeast production from high level waste sugar streams and other sugar sources- assuming 30% of yeast produced in 2010 (10 million #/yr.) is produced by Purdue-DOE technology.

This results in the production of 4.5e7# yr. of yeast product.

a) Energy savings over conv. tech. (0.3 from 1.6 KW-h/#)---> 2.0 x 10^{8} BTU/yr.

b) Energy saved from not treating sugars as waste @ 1KW-h/1 # BOD ----->3.5 x 10^{11} BTU/yr.

6. Utilization of Purdue-DOE technology for yeast production form low level BOD waste water from food processing plants assuming a waste water discharge of 100,000 gal/day @ 20,000 ppm BOD reduced to 5,00 ppm from 500 plants nationwide leads to the elimination of 4.6 x 10^{9} # of BOD per year in the year 2010.

   a) Energy savings over treating these wastes @ 1 KW-h/#BOD -----> 1.6 x 10^{13} BTU/yr.

   b) Production of 1.15 x 10^{9} # of fodder yeast worth $0.05/# or $54,750 income per plant.

7. Utilization of Purdue-DOE extractive fermentation for lactic acid from waste sugars. Energy use in lactic acid is largely due to purification costs. We are attempting to reduce these costs with an extractive system, however, we are not far enough along to begin to quantify the energy savings.

Economic Attractiveness

The Purdue ethanol processes offer a lower capital method for ethanol production. Capital costs for an add-on type arrangement for a waste sugar generator to add an ethanol production unit to his facility are estimated at only $1.00 per annual gallon at the 250,000 gal/yr. scale. Labor and operating costs are also substantially reduced. Thus we feel that the Purdue process may make it feasible to have many smaller ethanol facilities rather than be forced to the 10-40 million gal/yr. scale by economic of scale which is the current condition for batch ethanol production from corn/starch streams.

Preliminary design calculations on yeast production from both high and low level substrate streams are also encouraging. Again, the movement towards a continuous process, the reduction of energy needs, and the reductions in capital cost should make the Purdue yeast process very competitive for yeast production. We feel that there is a good possibility the use of centrifugal yeast harvesters can be eliminated for the production of yeast from high level substrates, resulting in a substantial reduction in plant complexity and cost. We determined an ROI of 90% for a process producing 1.5 tons/day of dried mineral yeast from concentrated whey permeate.

Preliminary Cost Estimates

			(sub totals)	
1. Building		**$220,000**		
2. Receiving				
Syrups	Silo	$55,000		
	oil recov	$10,000		
	sludge filt	$25,000	**$90,000**	
Solids	warehouse	$50,000		
	mixing tank	$55,000		
	starch cook	$115,000	**$220,000**	
3. Fermentation				
Cascade	ferm	$60,000		
	dist/dehyd	$125,000	**$185,000**	
CSRS/SAE	ferm	$60,000		
	SAED	$160,000	**$220,000**	
CSRS #2		$220,000	**$220,000**	
4. Yeast				
AYR		$85,000		
centrif.		$79,000		
yeast dryer		$45,000		
bagging		$40,000	**$249,000**	
5. Steam		**$65,000**		
6. Cooling water		**$180,000**		
7. Lab Equip		**$85,000**		**TOTAL Bare E $1,514,000**
8. Engineering (15% equi		**$227,100**		
9. Instru/Cntrl (18%)		**$272,520**		
10. Piping/pumps (35%)		**$529,900**		
11. Electical (7%)		**$105,980**		
TOTAL		**$2,869,500**		

Preliminary Cost Estimates

		Phase 1	Phase 2	TOTAL
1. Building		$220,000		$220,000
2. Receiving				
Syrups	Silo	$55,000		
	oil recov	$10,000		
	sludge filt	$25,000		
Solids	warehouse	$150,000		
	dry silo		$65,000	
	mixing tank	$55,000		
	starch cook		$115,000	$475,000
3. Fermentation				
Cascade	ferm	$60,000		
	dist/dehyd	$125,000		
CSRS/SAE	ferm	$60,000		
	SAED	$160,000		
CSRS #2			$220,000	$625,000
4. Yeast				
AYR		$85,000		
centrif.		$79,000		
yeast dryer		$45,000		
bagging		$40,000		$249,000
5. Steam		$65,000		$65,000
6. Cooling water		$180,000		$180,000
7. Lab Equip		$85,000		$85,000
8. Eth. Storage/Load out		$85,000		$85,000
9. Total Bare Equip		**$1,364,000**	**$400,000**	**$1,764,000**
8. Engineering (15% equip		$204,600	$60,000	$264,600
9. Instru/Cntrl (18%)		$245,520	$72,000	$317,520
10. Piping/pumps (35%)		$477,400	$140,000	$617,400
11. Electical (7%)		$95,480	$28,000	$123,480
TOTAL		**$2,607,000**	**$700,000**	**$3,307,000**

2. Pilot Scale Process Development

A major goal of the Industrial Waste Program of DOE's Office of Industrial Technologies is to move technology from theoretical models / lab bench practice/ to pilot scale and industrial practice. Industry generally needs to see a working pilot scale unit before investing in new technology. Thus, over the coarse of this project, we have built 6 pilot scale 'new technology' pilot plants. A brief description of these units follows, and more details are provided in the Appendices.

2.1 50 Liter Immobilized Cell Reactor Separator (ICRS) for Development Demonstration of Fermentation and Separation Technologies

A 50 liter immobilized cell reactor was designed and built at Purdue University. This unit consists of 6" ID stainless steel columns. The unit was first designed to run with feed adsorption and product condensation as shown in Figure 2.1, but was later modified to test the solvent absorption/ extractive distillation (SAED) process for ethanol recovery from dilute vapors. This unit was operated for a period of over 60 days continuous operation using concentrated acid whey permeate provided to the project by Dairy Farm Products Inc. of Orville Ohio. A description of this project is given in Appendix 2.1. This successful project was followed up by the design of a full scale unit for DFP, but the DFP plant went out of operation due to labor disputes before the project could be implemented.

2.2 1000 L ICRS for Ethanol from Acid Whey Permeate

A 1000 liter ICRS unit was built in 1987-88 using a newly developed spiral wound packing matrix. A schematic of the unit is shown in Figure 2.2a, and a photo in Figure 2.2b. A 6 inch ID high efficiency distillation column was built and tested as shown in Figure 2.2 c to concentrate the ethanol from this unit. The unit was operated at a Ft. Wayne IN dairy for a period of about 1 year. Problems with non-sterile feed (raw acid type whey from a cottage cheese operation), and leakage of air born contaminants into the reactor caused performance of the unit to deteriorate over time. Our experience with the 50 L ICRS indicated that good long term performance could be demonstrated, but the high initial load of lactic bacteria in the raw whey fed to the 1000 L ICRS at the dairy proved to be difficult to control with a hot hold type pasteurization /sterilization procedure. This unit showed us the necessity for both maintaining a sterile feed, and the need for air-tight construction /fabrication of the entire unit to prevent contamination of the reactor.

2.3. 7,500 L ICRS for Ethanol from Concentrated Whey Permeate

A 7,500 liter unitized ICRS/ absorber was constructed in 1991-92. A schematic of the unit is shown in Figure 2.3a, and a description of the unit is given in Appendix 2.3 This unit combined a novel spacerless packing for the ICRS (jointly developed by M.C. Dale, W. Harris of Kenyon Enterprises, and R. Hinner of Merrill I&S) and an absorber unit. Gas flows through the ICRS and

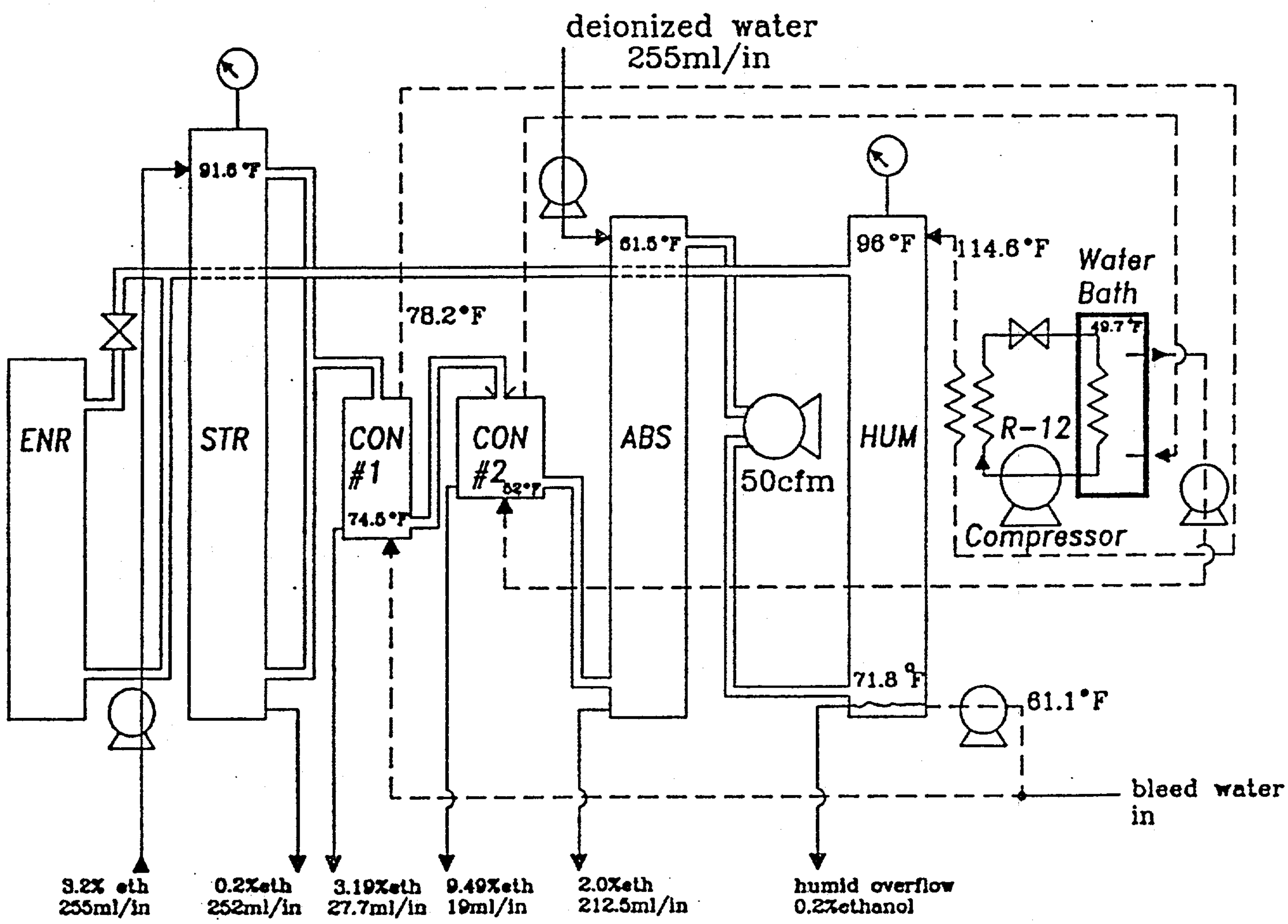


Figure 2.1 The 6" OD 50 Liter Pilot Plant Design

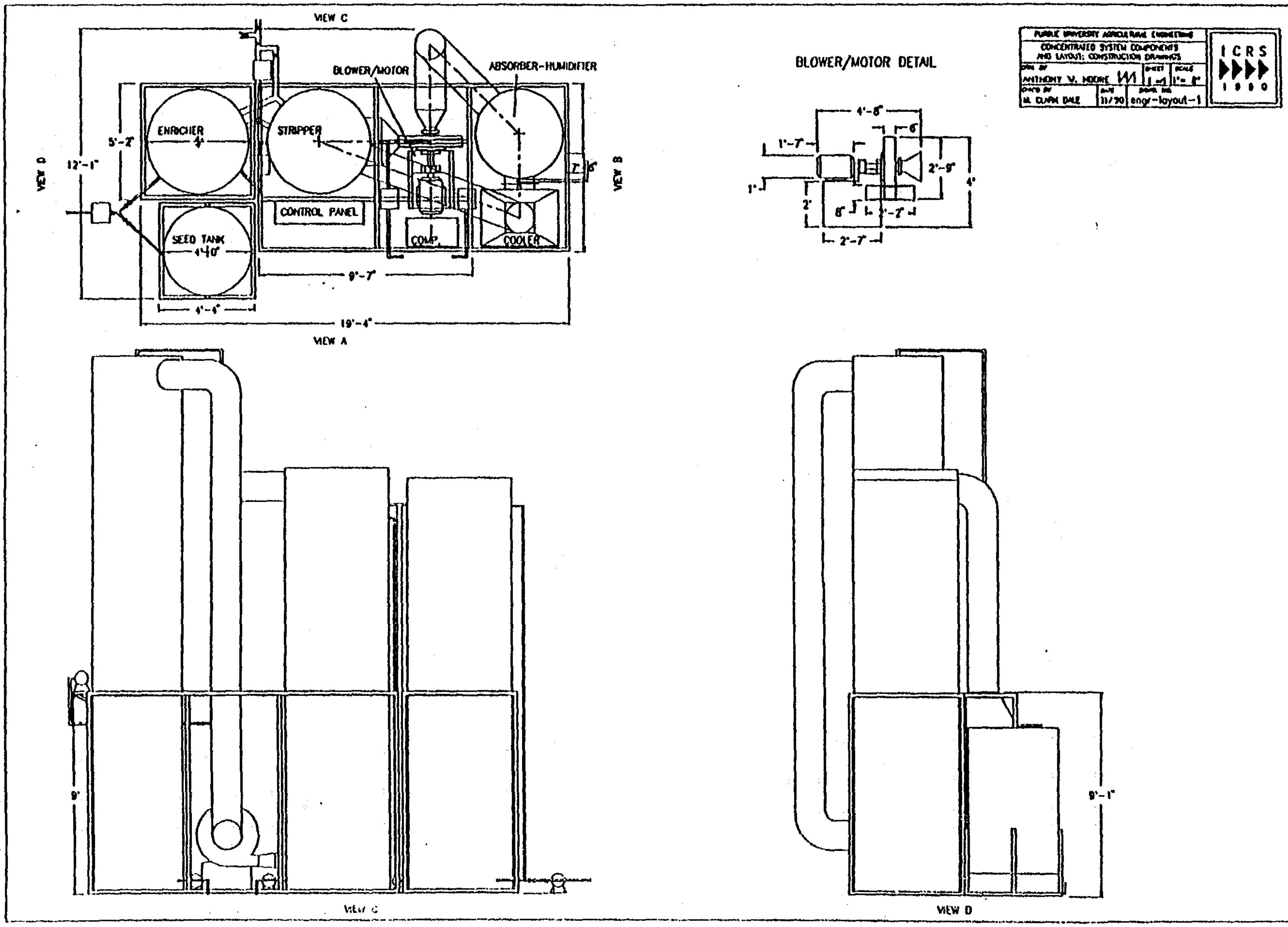


Figure 2.2 a. Design Prints for the 1000 L ICRS Pilot Plant

ystem.

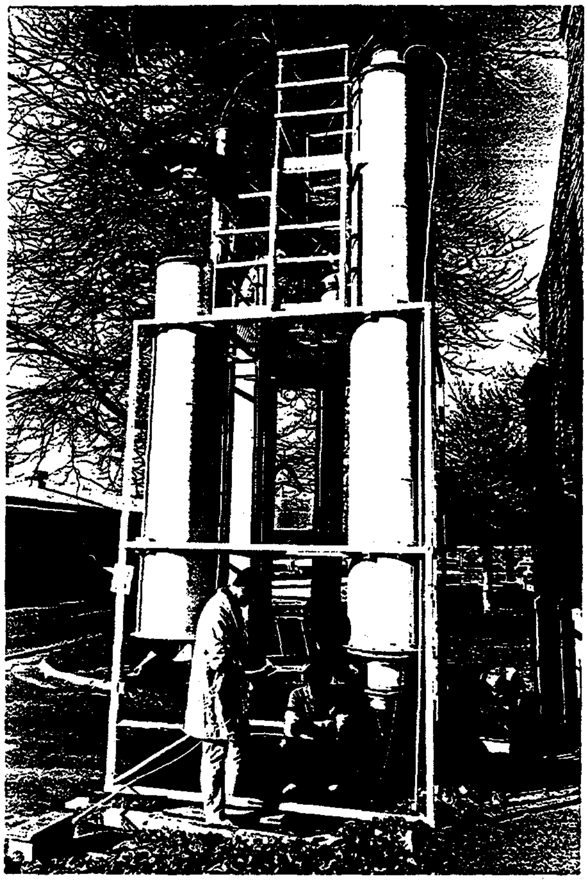


Figure 2.2 b. A photo of the 1000 L ICRS Pilot Plant befoe installation on site.

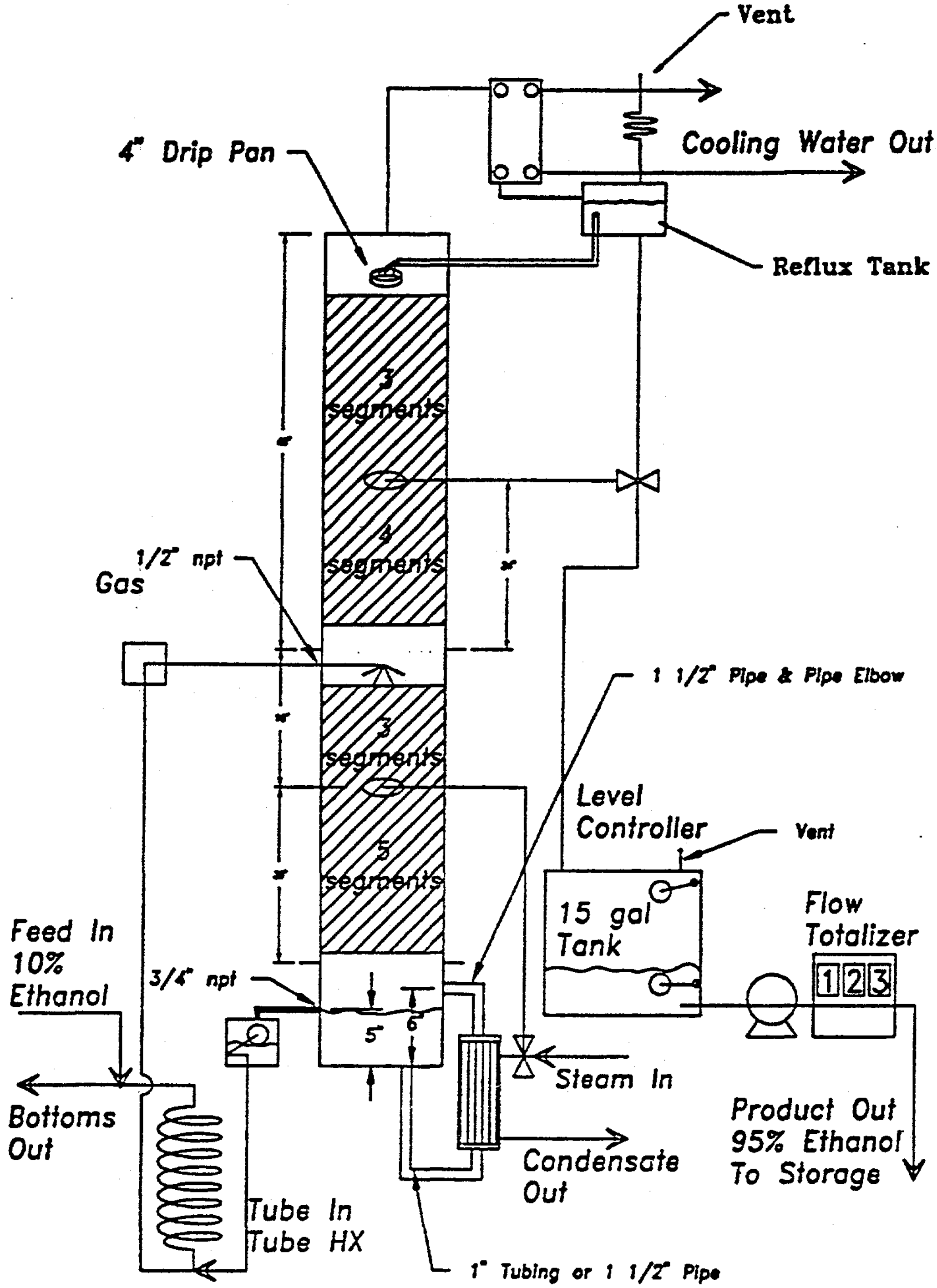


Figure 2.2 c. Design Prints for the 6" OD Distillation Column used with the 1000 L ICRS

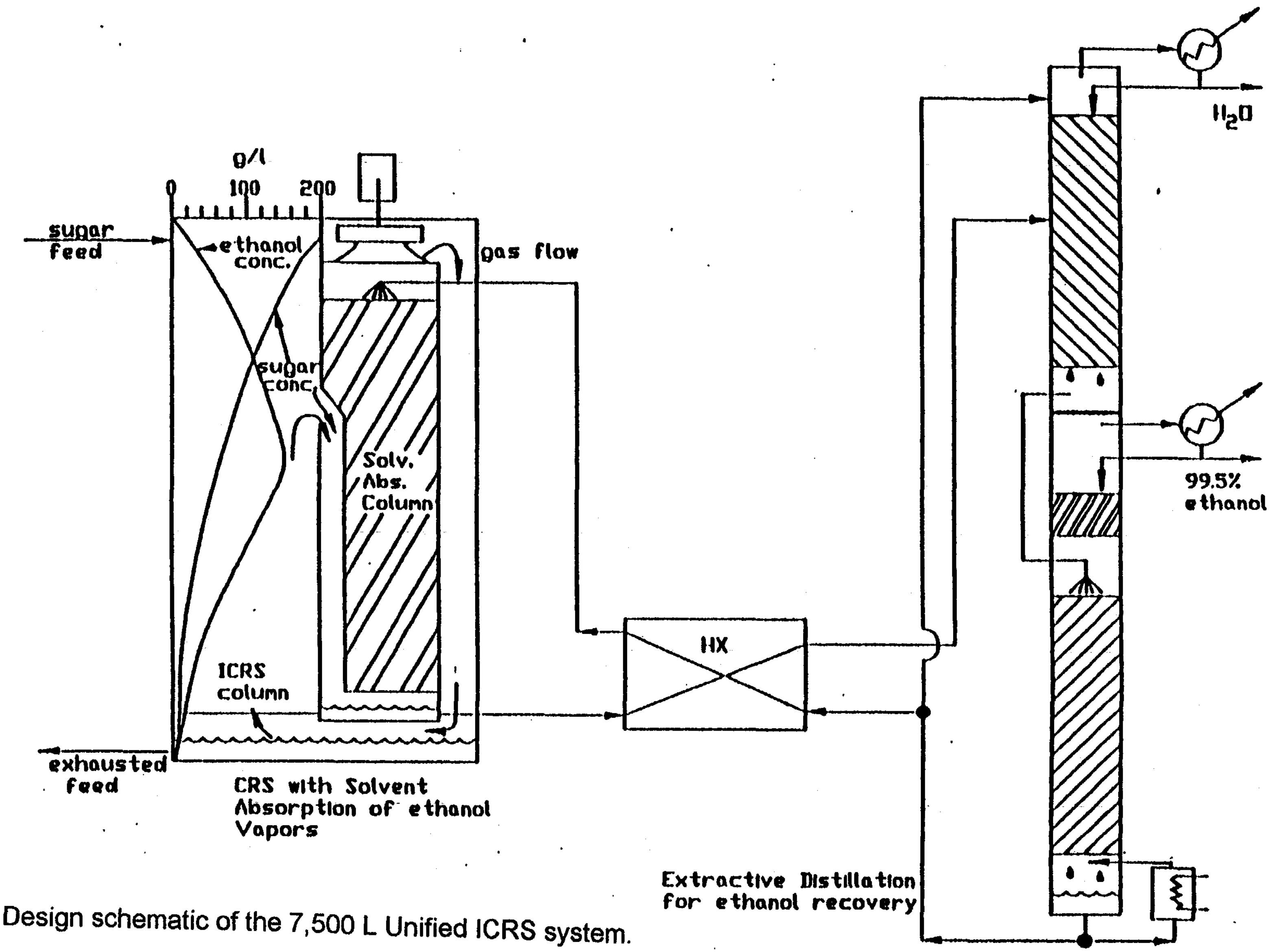


Figure 2.3 a. Design schematic of the 7,500 L Unified ICRS system.

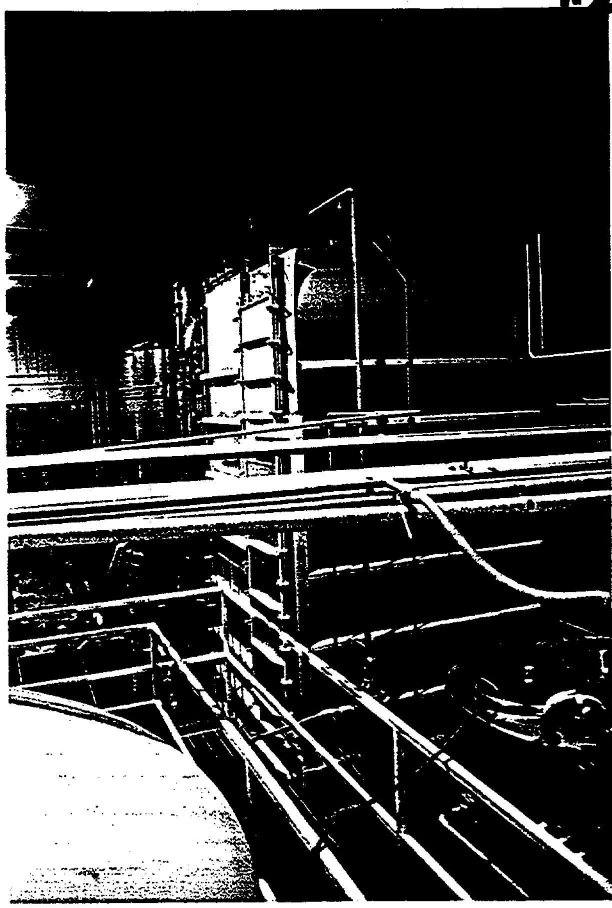


Figure 2.3 b. A photo of the 7,500 L Unified ICRS/ Absorber on site at PRI.

absorber were achieved with an internally mounted blower, and internal ducting. These modifications allowed the unit to be built with no external ducts for the circulating gas stream, minimizing the chances for external contamination of the fermentation matrix with unwanted organisms. The unit was operated at the PRI site for about 6 months in 1992. The reactor ran well for a short period of time, but bacterial contamination of the feed (concentrated sweet whey permeate) from a near-by cheese plant led to difficulties in stable long term operation of the unit. The supply of whey permeate was then terminated as the cheese plant closed its operation. The site host facility was not willing to invest in a system for feed sterilization, either heat or membrane based, or for a well as a well designed (sterile) seed inoculation system. Both of these are prerequisites for long term successful operation of a ICRS system. We were fairly pleased with the design and fabrication of the unitized ICRS/ absorber system.

2.4 200 L Immobilized Yeast Reactor for High Levels of Yeast from Dairy Waste Whey Streams

A 200 liter immobilized aerobic reactor was constructed by Kenyon Enterprises to test the ability of a spacerless packed matrix to convert high levels of sugars to yeast biomass. A photo of this unit is shown in Figure 2.4. This reactor allows excellent oxygen transfer at a low pressure drop. The same sort of analysis performed by Dale et al (1990) for ethanol production by yeast would apply, except that the driving force is adsorption of oxygen into the immobilized cell matrix rather than stripping of a volatile product such as ethanol. The work performed by Harris in Wisconsin, and Dale & Moelhman at Purdue indicated that oxygen transfer was probably limiting conversion efficiencies of sugars to biomass. This work was co-sponsored by the Wisconsin Milk Marketing Board, Kenyon Enterprises, and BPI. The system ran well for extended periods of time and has definite commercial potential for the production of yeast. Contamination of the reactor by film forming molds is a concern, so we suspect that a fed- batch type operation, where the system is sterilized on a regular time interval might be the most appropriate commercial design.

2.5 24,000L Continuous Stirred Reactor Separator (CSRS) for Ethanol from Waste Candy/ Starch

A 24,000 liter, 4 stage CSRS was fabricated from 304 stainless by Lamont Sign Co. A schematic of this unit is given in Figure 2.5, and the unit design and performance is discussed in Appendix 2.5 This system has been successfully operated for a period of over 24 months to date of almost continuous 24 hour/day operation. Feed to the system has been candy rinse waters and waste starch streams from various food processors. The system has shown a remarkable resistance to bacterial or wild yeast contamination over this period of time. The CSRS was inoculated with a strain of flocculent yeast, with the intent of building up a cell density of the flocculent yeast 'flakes' or 'pellets'. These pellets were noted occasionally but the build-up to a 33% settled yeast density was not noted. The ability to control the stirring rate was lost due to electrical problems which may have caused the turbulence to be too severe for good flocculating of the

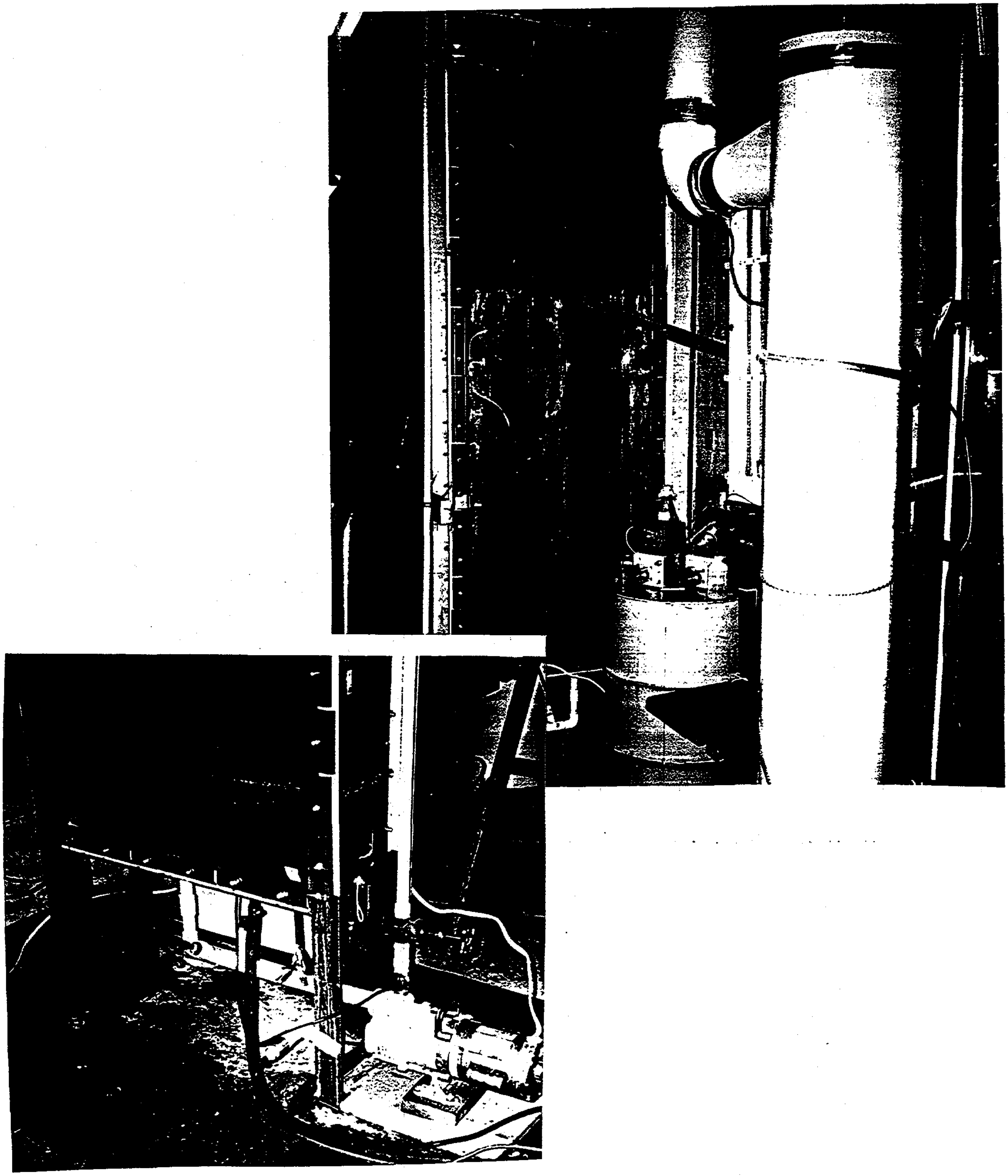


Figure 2.4 Photographs of the 200 L Aerobic Yeast Reactor.

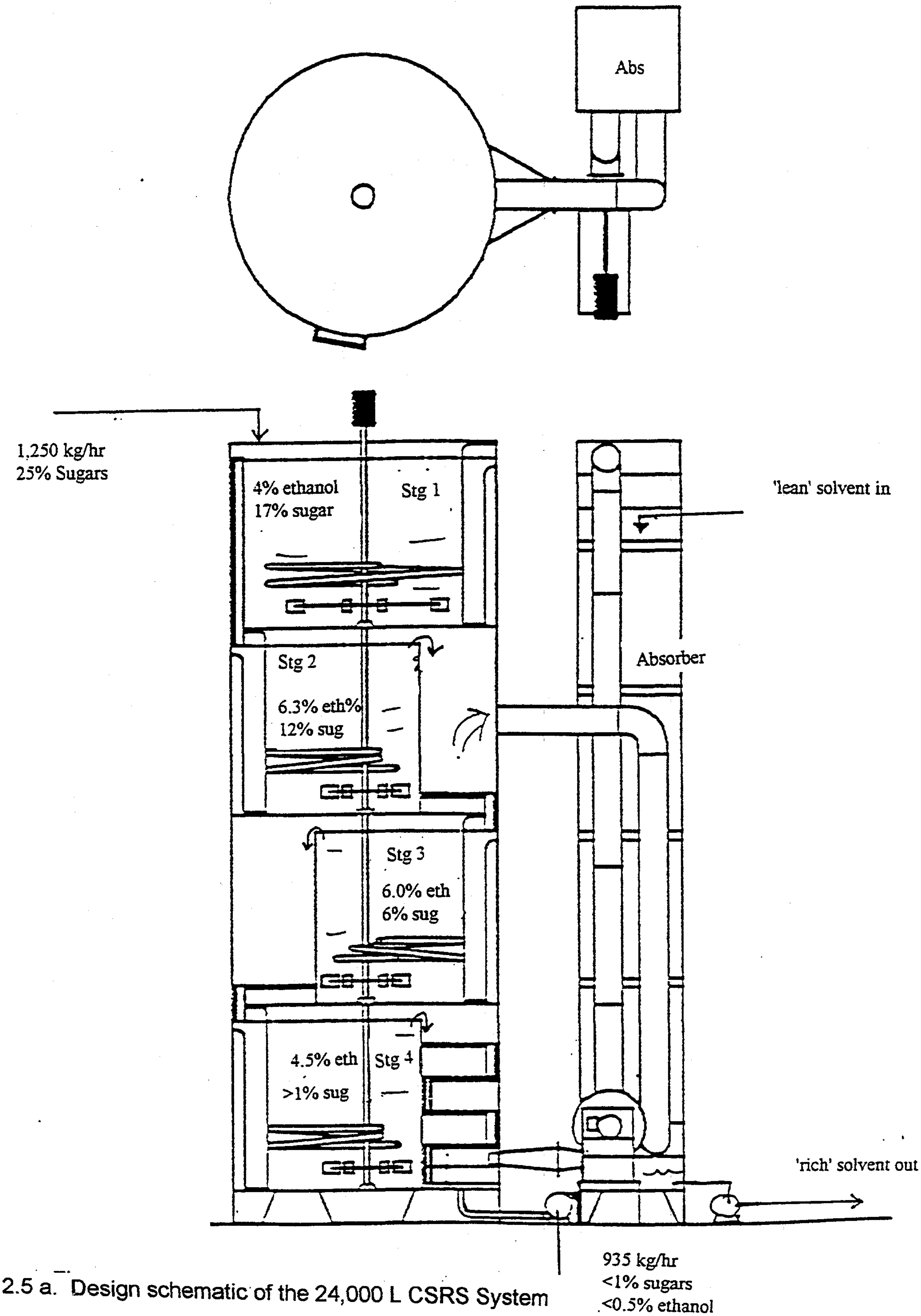


Figure 2.5 a. Design schematic of the 24,000 L CSRS System

Figure 2.5 b. Photograph of CSRS on site at PRI site.

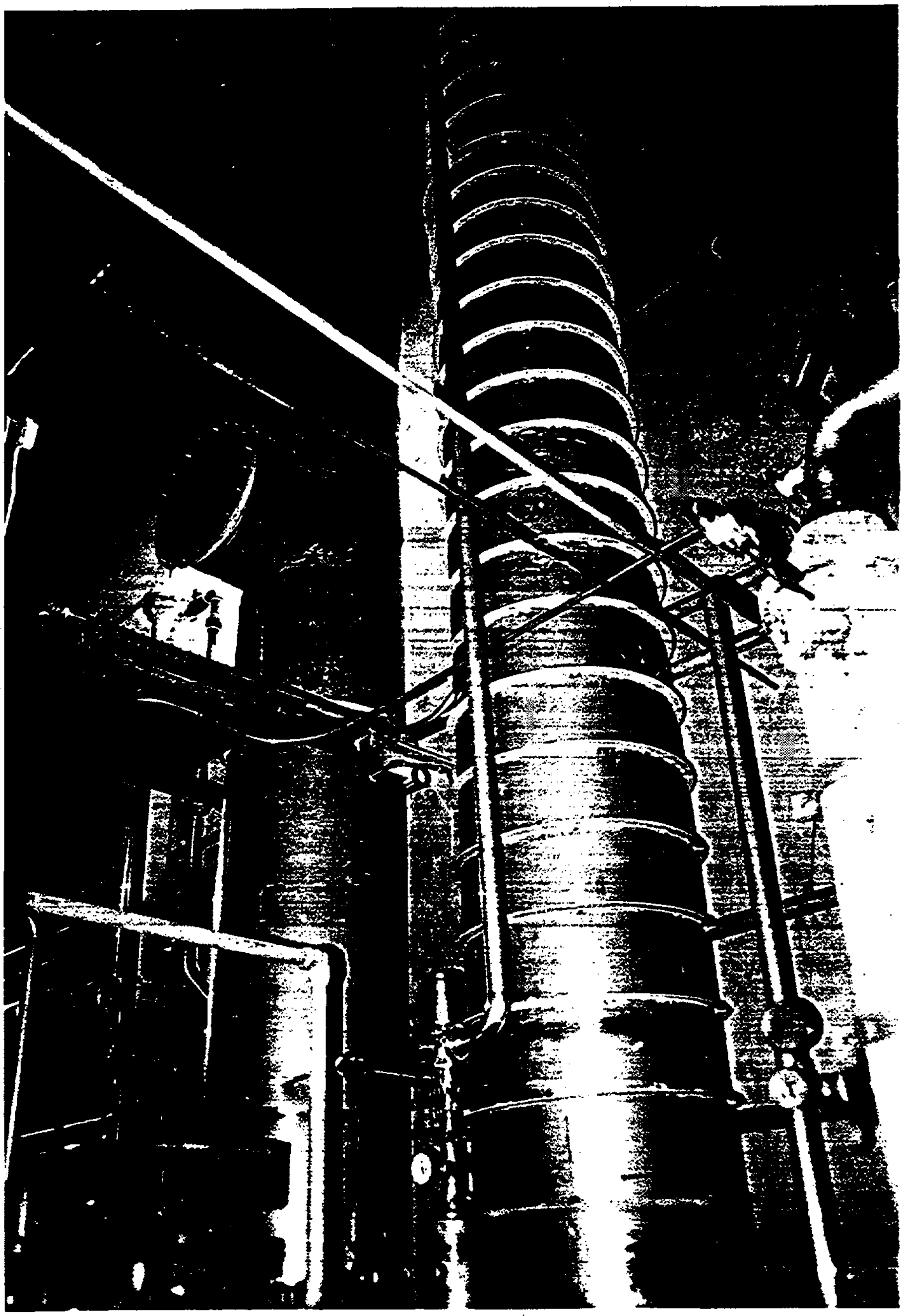


Figure 2.5 c. Photo of Extractive Distillation Columns for Solvent Absorption System at the PRI Site.

yeast. This reactor, with its ability to handle non-sterile feeds, would seem to be the most 'appropriate' technology for low technology operations as per the Permeate Refining plant in Hopkinton, IA.

2.6 55 L 6 stage CSRS for Ethanol from Cellulosics.

A 55 liter 6 stage continuous staged stirred reactor separator (CSRS) was built in 1996 to develop a dual culture process for conversion of cellulosics to ethanol. The basic design scheme is to successively ferment the hemi-cellulose (xylans) followed by celluose (glucans) in a multi-stage reactor. Approximately half of the reactor volume will be devoted to each SSF fermentation using conventional (non-genetically engineered) yeast strains. The fabrication costs were provided by Indiana Biomass Energy and BPI. A photo of this unit is shown in Figure 2.6.

2.7 32,000 L Aerobic Yeast Reactor for Yeast from High BOD Distillery Waste Water

A 32,000 liter draft tube aerobic yeast reactor was built with at the PRI site. This unit has the purpose of growing yeast on the fermentation bottom water, reducing lactic acid, glycerol, and other trace components of the bottom water. This pilot plant is following up on the work of Dale et al (1992) and Salicetti (1996). A sketch of the unit is shown in Figure 2.7 This unit has the basic goal of reducing the BOD of the bottoms water by about 80 to 90% allowing a high level of back set of the bottoms water back into the fermentation process.

3. Laboratory Studies

A number of processes have been worked on at Purdue University over the course of this project. Basic research on fermentation processes have focused on improving fermentation of waste substrate via:

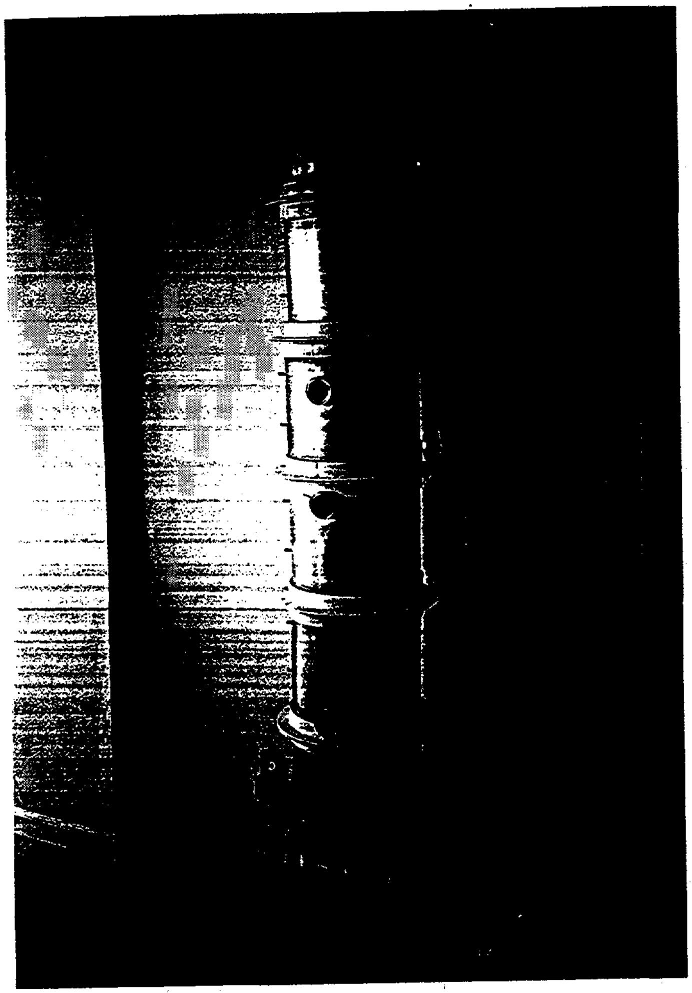
1) maintaining a high cell density in the reactor by immobilizing cells, or using flocculated cells

2) incorporating removal of inhibitory products from the fermentation broth during the fermentation

3) developing continuous fermentations so as to reduce tankage, cleaning, and operating costs

4) incorporating simultaneous saccharification of feed polymers such as starch and cellulose to reduce substrate inhibition of enzymes or fermentations.

5) develop models for predicting and understanding the kinetics of fermentations as affected by environmental, nutritional, and product levels, and use these models to improve the reactor design so as to maximize long term performance of the reactors.



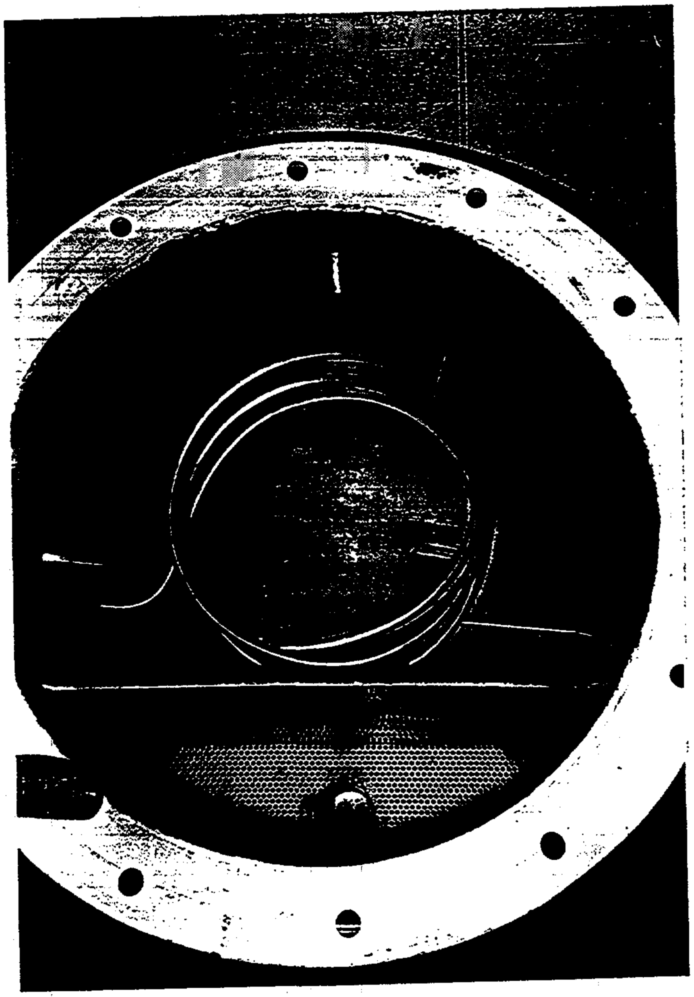


Figure 2.6. Photos of the 55 L CSRS for Cellulose Conversion to Ethanol.

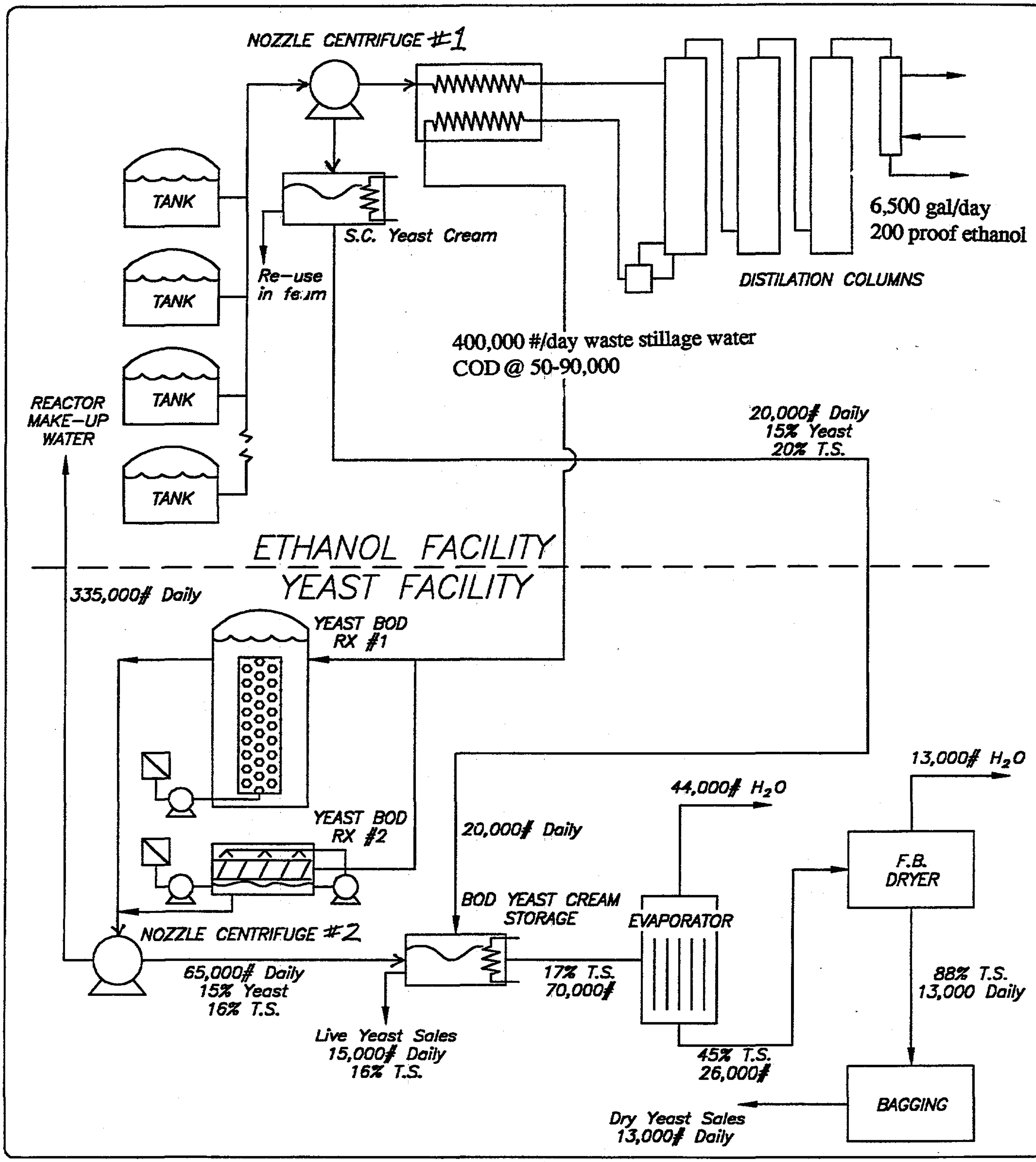


Figure 2.7 Aerobic Yeast Reactor System for BOD Reduction from a Distillery.

No.

Scale: NONE

Date: 10/26/94

Issue: M. Mark Dale

Drawn By: BLK

Purdue University
Agricultural Engineering
AGEN • West Lafayette, IN 47907

3.1 a. Lactic acid production

Lactic acid may have a major role to play as a monomer for bio-derived bio-degradable plastics. Poly lactic acid (PLA) also has been noted to have beneficial effects on plant growth and could thus play a role in slow release chemicals associated with crops such as herbicides and insecticides. Over the course of this project, K.V. Venkatesh worked on modeling the lactic acid fermentation of sugars and incorporating extractive fermentation for lactic acid removal from the broth. A membrane was suggested to separate the fermentation broth from the media due to the toxic nature of solvents with a high affinity for lactic acid. This work has been continued over the last several years by N. Burgos. A presentation summarizing this work is included in Appendix 3.1a.

3.1 b. SSF of Cellulose to Lactic Acid

The use of cellulose as a substrate for lactic acid was suggested and modeled by K. Venkatesh. Simultaneous saccharification and fermentation (SSF) reduces the glucose inhibition of the cellulase enzymes. This work is summarized in Appendix 3.1 b.

3.2 Diacetyl Production w/ Lactic acid

Diacetyl is a flavor compound with a characteristic "buttery" taste. A variety of volatile and non-volatile flavor compounds were evaluated for possible study as a Ph.D. project by W. Lee. He selected diacetyl due to the combined high volume and high price associated with this flavor compound.

Diacetyl production as a secondary metabolite during the lactic acid fermentation was studied, and it was found that high levels of diacetyl could be generated, and simultaneously gas stripped from a stirred reactor. High concentrations of diacetyl were recovered in the condensate after cooling the stripping gases. This work is summarized in Appendix 3.2. This work would seem to have commercial applicability.

3.3 a. Anti-fungal compounds from Continuous Plant Cell Culture

The use of some of our ICRS type packing methodology as applied to plant cell tissue culture has been explored by a recent Ph.D. student, H.J. Choi. A continuous plant cell reactor was developed which gave off the desired compound, gossypol, into the fermentation media as the media was circulated through the reactor. Combining elicitation, i.e. methods to stimulate the production of the desired compound, with permeabilization of the cell wall, allowed high levels of the anti-fungal agent to be continuously generated and excreted into the fermentation media. This work is detailed in Appendix 3.3 a.

3.3 b. Taxane compounds from Continuous Plant Cell Culture

The extrapolation of Mr. Choi's work to taxane production by species of the *Taxus* family is currently being evaluated. A *Taxus* plant species which is characterized by excreting taxanes (as compared to intracellular accumulation) was chosen for study. Plant cell reactors were constructed using a number of various flow and packing methodologies. Some reactors were operated at Purdue, and others at BPI's laboratory. This work may allow the continuous production and harvesting of potent anti-cancer agents, taxol / taxanes from immobilized plant cell reactors. Work on optimizing the media for taxane production is still underway.

3.4 a. Yeast from Low Levels of Lactic acid

Our efforts to convert the lactose fraction of acid type whey to ethanol led us to associated efforts to use/ eliminate other low level components of the whey. Lactic acid (LA) is produced during the production of cottage cheese by lactobaccilus cultures. Acid levels of up to 0.5% are regularly noted. There is also a small amount of LA given off by yeast during fermentation. In this portion of the project, we were attempting to utilize secondary components in the whey to 1) reduce the BOD of the 'bottoms water', and 2) produce a valuable by-product, yeast or single cell protein. Yeast strains were screened for ability to metabolize LA aerobically, and reactors for LA utilization built and operated. Some of this work is described in Appendix 3.4 a.

3.4 b. Yeast from Multiple Low Level Substrates

The work described in 3.4 a, and Appendix 3.4a was followed by two further efforts, 1) lab and industrial scale implementation of Aerobic Yeast Reactors, and 2) a detailed theoretical study of growing multiple yeast strains on multiple substrates was undertaken by L. Salicetti as a Ph.D. project. An overview of Dr. Salicetti's work is given in Appendix 3.4 b.

3.4 c. Yeast from high strength waste syrup streams

A cooperative effort between Purdue, Kenyon Enterprises, Merrill Iron & Steel, and BPI investigated the possibility of using an immobilized matrix of yeast for high density yeast production. Lab scale reactors were operated at Purdue by Dale, Moehlman and Zhao, and a 200 L pilot plant was built by KE and Merrill I&S. Lab scale experiments indicated that up to a 25% sugar/ whey lactose solution could be converted to 10-11% yeast product. Contamination by molds was noted to be a difficulty in the scaled-up pilot plant.

3.5 a. Gluconic Acid from Waste Glucose streams

The possibility of providing as gas phase nutrient (oxygen) to immobilized cells using the packing methodology developed during this project was explored. In the gluconic acid fermentation, glucose is oxidized to gluconic acid by *gluconobactors* in the presence of oxygen. Good rates and long term stability were noted in lab scale experiments using immobilized whole bacterial cells. Some details of this work are given in Appendix 3.5 a.

3.5 b. Acetic acid from Ethanol

In a similar reactor as discussed above (3.5 a) immobilized *acetobactors* can be used to oxidize ethanol to acetic acid. A small acetator reactor was operated for several months using immobilized cells. Good performance was noted.

3.6 a. The CSRS for SSF of Cellulose to Ethanol

A successive dual culture for the conversion of hemicellulose (xylans) and cellulose (glucans) to ethanol has been proposed and is being developed currently by BPI (Dale, 1996) as a follow-on project. This is a spin-off effort of this project and we are hopeful that this technology will have a positive impact on moving the US towards a renewable energy basis in the next 5 to 10 years.

3.6 b. The Xylan Pretreatment Process for Cellulosics

Xylan Inc. has developed a thermo-mechanical-chemical process for the delignification of cellulosics. Xylan cooperated with this DOE project in the hopes that this pretreatment technology, developed with the fermentation technology which forms the basis for this project, could make a significant improvement in current technology for enzymatic hydrolysis and fermentation of cellulosic to ethanol. A description of our evaluation of this technology is given in Appendix 3.6 b.

3.6 c. Xylose Fermentation to Ethanol

There is a growing effort to develop (genetically engineer) strains of microbes able to ferment both xylose and ethanol. Ingrams group at the U. of Florida has developed strains of E. coli, NREL in Golden CO has developed a strain of Z. mobilis, and N. Ho of Purdue's LORRE is developing a Saccharomyces yeast. In our work, however, we are looking at methods of using currently available non-engineered strains of yeast, but completing the fermentation via improvements in reactor design rather than microbial design. The fermentation of xylose to ethanol was studied previously at Purdue by Slinninger (1992). We followed up this work, evaluating the ability of various strains to ferment xylose and xylose glucose mixtures. This work is summarized in Appendix 3.6 c.

3.7 Immobilized Cell Reactor Studies

The use of immobilized cells for the production of ethanol from sugars was studied by Dale, Chen, and Lin. This work is summarized in Appendix 3.7. We determined that a stable, steady state live cell population could be maintained in an immobilized cell reactor using adsorbed cells, only if cells were desorbed or underwent lysis after death. The actual steady state live cell density of the population was described mathematically by Dale et al (1990), and matched data shown by Chen et al (1990). Nutritional needs were also investigated. Due to the mature nature of the immobilized cells, it was found that nutritional need could be reduced by a factor of over 10X as compared to batch type operations.

A similar set of studies on using an ethanololic bacteria, Z. mobilis was investigated. We found that extremely high rates of fermentation could be attained using this bacteria. Residence times as low as 40 minutes could produce a 10% ethanol fermentation broth (Lin et al. 1990).

3.7 a. Concentrated Molasses and/or Sucrose to Ethanol with the ICRS

The use of very concentrated sugar feeds can be contemplated in the ICRS and CSRS as the normal limitation of final ethanol tolerance of the organism is lifted. In normal batch or continuous fermentations, if a 11% ethanol concentration is the maximum that the yeast can tolerate, a feed concentration of 23% (assuming a conversion efficiency of 0.48 g/g) would be fed. In the ICRS and CSRS however, this limit is lifted as ethanol is separated from the broth as it is formed. The new limits on feed concentration have to do with osmotic inhibition of the feed sugar solution. In some tests with an osmo-tolerant strain of yeast, up to 45% sucrose solutions were fermented, and 35% molasses. Details of this work are given in Appendix 3.7 a.

3.7 b. Extreme thermophilic Conversion of Lactose to Ethanol with Immobilized Cells

The use of extreme thermophiles was evaluated for ICRS type reactors. This sort of application would allow ethanol to be stripped from the reactor with little or no added gas, as, if the reactor is operated at 65 C, the vapor pressure of the ethanol/water solution will be near boiling. A series of tests on extreme thermophilic fermentation of lactose was completed by D. Lineback as a Ph.D. project. These tests showed that although the concept is practicable, the strains tested produced a variety of products if the feed strength was over 20 g/L lactose. Some details of this study are given in Appendix 3.7 b.

3.7 c. The Acetone Butanol Ethanol (ABE) Fermentation in an ICRS

The application of the ICRS technology as developed for ethanol production was extended to the acetone/ butanol/ ethanol (ABE) fermentation by a Ph.D. student, C.H. Park. This work demonstrated that high levels of C. acetobutylicum could be immobilized, and that the solvents could be successfully stripped from the immobilized cell reactor. Keeping the cells in the solvent producing mode is a difficulty which constrains further development of this effort. Papers describing this work are given in Appendix 3.7 c.

3.8 Flocculent yeast for High Cell Density Fermentations

Fermentation rates generally increase linearly with cell concentration. A fermentation operating with 2 time the cell density of a basal fermentation will be about 2 times as quick as the basal fermentation. There are two basic ways to maintain a high cell density in a fermentation, 1) recycle the cells after a centrifugal or membrane separation process, or 2) immobilize the yeast in some manner within the fermenter. Immobilization can take the form of adsorption, entrapment, or agglomeration into flocculent particles. In this portion of the project, we evaluated several strains of flocculent yeast for high density stirred type ethanol reactors. We found that settled cell densities averaged 50 g/L and settled cell levels of between 33 and 66% allowed fermentations at 20-25 g/L. These densities allow a complete fermentation in 24 hours for batch conversion of 22% sugars. This work is summarized in Appendix 3.8.

3.9 Osmolality model for Inhibition of Ethanolic Fermentation

The work competed on highly concentrated feeds for ICRS type fermenters caused us to begin a study on the effects of osmolality on various strains of yeast. These studies resulted in a model that basically suggests that both product (ethanolic) and substrate (sugars) inhibition of yeast are largely an osmotic phenomena. This model has far reaching ramifications in the modeling and design of fermentations. It also serves as an excellent and easy method to allow the effects of multiple sugars, salts, and other inhibitors to be approximated. A paper on this work is given in Appendix 3.9.

3.10 Fermentation of Permeate Mother Liquor to Ethanol

A spin-off of our work on cascade fermentations, and the development of the osmolality model developed during this project (Appendix 3.9) is the fermentation of permeate mother liquor by an strain of *K. marxianus* yeast which were adapted by BPI's lab to ferment lactose in high salt environments. Permeate mother liquor is the residue after concentration of whey permeate to beyond the solubility point of lactose, crystallization of the lactose, and centrifugal separation of the lactose crystals. The remaining liquid is comparable to the molasses left in the cane sugar process. The residue is termed 'permeate mother liquor' (PML) and has a composition of 50-60% lactose, 20-30% salts, and some proteins.

Our osmolality model shows the effects of higher salts, limiting cell growth and final ethanol. The model also allows us to predict optimal operating conditions, and maximal concentrations of feed and final ethanol based on the inlet salt levels. This work led to the industrial fermentation of PML at a site in Minnesota. A description of this work is given in Appendix 3.10.

3.11 SSF of Raw Starch to Ethanol in a CSRS

The possibility of converting raw starch to ethanol is an idea developed during this project. Work on this project was supported by BPI and the Great Lakes Governors Council. The basic concept is to save energy and capital expenses by elimination of the high temperature 'liquefaction' cook of the starch slurry, which is the industry standard method to convert starch granules to dextrins. This process requires that the starch slurry be heated to 100-120 C for a period of time in the presence of alpha amylase. The dextrins are then enzymatically converted to glucose and then fermented. A process for no-cook conversion of starch was conceptualized and proven on the lab bench scale. We found 85-95% conversion of the raw starch could be attained, as compared to 95-98% conversion during the standard cook conversion process. We feel that this process may have considerable industrial promise when the system is run in conjunction with a animal (cattle) feeding operation. A summary of this work is given in Appendix 3.11.