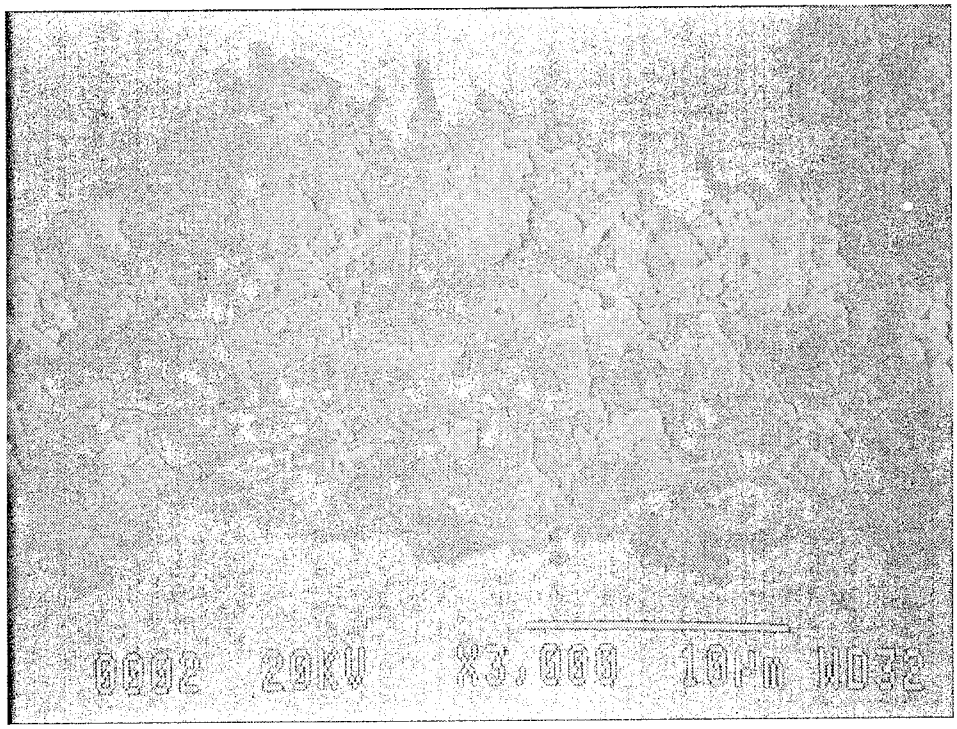
Calcium Magnesium Acetate at Lower-Production Cost: Production of CMA Deicer from Cheese Whey

PUBLICATION NO. FHWA-RD-98-174

APRIL 1999

PB99-148991



U.S. Department of Transportation
Federal Highway Administration

Research, Development, and Technology
Turner-Fairbank Highway Research Center
6300 Georgetown Pike
McLean, VA 22101-2296

New York State Energy Research and
Development Authority
Corporate Plaza West
286 Washington Avenue Extension
Albany, NY 12203-6399

FOREWORD

This report documents a study whose purpose was to investigate methods to produce low-cost calcium magnesium acetate (CMA), an alternate chemical deicer to commonly used chloride deicers. A fermentation process using cheese whey as a starting materials was developed to generate acetic acid, the cost controlling component in the production of CMA. An energy-efficient solvent extraction process was also developed to separate and recover acetic acid from the fermentation process. CMA produced using acetic acid from these processes had good ice penetration properties. Cost analyses from these processes showed that CMA could be produced at a cost varying from $204-$328/ton. Detailed process evaluation and cost analyses are given in the report.

T. Paul Teng, P.E.
Director, Office of Infrastructure R&D

NOTICE

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
FHWA-RD-98-174		

4. Title and Subtitle	5. Report Date
Calcium Magnesium Acetate at Lower-Production Cost: Production of CMA Deicer from Cheese Whey	April 1999
	6. Performing Organization Code
7. Author(s)	8. Performing Organization Report No.
Shang-Tian Yang, Y.L. Huang, Zuwei Jin, Yan Huang, Hui Zhu, Wei Qin	
9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
The Ohio State University Department of Chemical Engineering 140 West 19th Ave., Columbus Ohio 43210	3E6A
	11. Contract or Grant No.
	DTFH61-93-C-00013 / 4161-ERTER-ER-95
	13. Type of Report and Period Covered
12. Sponsoring Agency Name and Address	Final Report 5/21/93-8/31/97
Federal Highway Administration, Turner-Fairbank Highway Research Center 6300 Georgetown Pike, McLean, VA 22101-2296 and New York State Energy Research and Development Authority (NYSERDA) Corporate Plaza West, 286 Washington Ave. Extension, Albany, NY 12203	14. Sponsoring Agency Code

15. Supplementary Notes

Contracting Officer's Technical Representative (COTR): Dr. Brian Chollar, HNR-20. NYSERDA Liason: Judy L. Jarnefeld. Industrial Sponsors: Kraft Foods, Brewster Dairy, Glitsch Technology Corp. Cooperating DOT's: CA, IA, MI, MN, MT, NE, NV, NM, NY, NC, OH, OK, OR, SD, TX, VT, WI; Cryotech Deicing Technology Program (Keith Johnson)

16. Abstract

Calcium magnesium acetate (CMA), a mixture of calcium acetate and magnesium acetate, is used as an environmentally benign roadway deicer. The present commercial CMA deicer made from glacial acetic acid and dolomitic lime or limestone is expensive compared with salt and other deicers. Also, a liquid potassium acetate deicer is used to replace urea and glycol in airport runway deicing. The goal of this project was to develop low-cost acetate deicers from cheap feedstocks, such as biomass and industrial wastes, via fermentation. A novel fibrous bed bioreactor was developed for fermentation to produce acetic acid from whey lactose by a co-culture of Lactococcus lactis and Clostridium formicoaceticum. The acetic acid yield from lactose in this fermentation was 90 percent, and the acetate concentration from the fed-batch fermentation was as high as 75 g/L. An energy-efficient solvent-extraction process was also developed to separate and recover acetic acid from the fermentation broth. Back-extraction or stripping with a base solution produced acetate salt at a high concentration,> 20 percent (weight per volume)(w/v), and simultaneously regenerated the solvent. Two alternative methods to produce low-cost acetate deicers from cheese whey were studied. CMA deicers produced from cheese whey by fermentation and extraction were tested for their acetate content and deicing properties. The CMA solid sample obtained from extraction of the acetic acid present in a dilute aqueous solution and then back-extracted with dolomitic lime to form CMA had about the same acetate content (70 percent acetic acid or 90 percent CMA) as that of the commercial CMA deicer. The sample from dried whey fermentation broth contained 50 percent acetic acid or 63 percent CMA, with the remaining solids being other organics and salts present in whey. Deicing tests showed that CMA samples from fermentation and extraction had an equal or slightly better ice penetration rate than that of the commercial CMA. Cost analysis showed that CMA can be produced at a product cost of $204-$328/ton ($224-$360/tonne), less than 30 percent of the current market price for the commercial CMA, for a plant size of 8400 tons (7640 tonnes) CMA per year. The lower CMA cost should dramatically increase CMA use in the deicing market. Scale up of these processes is feasible. Detailed process evaluation and cost analysis are given in this report.

Cover Photo: Scanning electron micrograph of C. formicoaceticum (long rods) and L. lactis (cocci) immobilized on the fiber surface in the fibrous bed reactor.

17. Key Words	18. Distribution Statement
Calcium magnesium acetate, deicer, fermentation, extraction, cheese whey, lactose, CMA.	No restrictions. This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	120	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

SI* (MODERN METRIC) CONVERSION FACTORS

APPROXIMATE CONVERSIONS TO SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
	LENGTH			
in	inches	25.4	millimeters	mm
ft	feet	0.305	meters	m
yd	yards	0.914	meters	m
mi	miles	1.61	kilometers	km
	AREA			
in^2	square inches	645.2	square millimeters	mm^2
ft^2	square feet	0.093	square meters	m^2
yd^2	square yards	0.836	square meters	m^2
ac	acres	0.405	hectares	ha
mi^2	square miles	2.59	square kilometers	km^2
	VOLUME			
fl oz	fluid ounces	29.57	milliliters	mL
gal	gallons	3.785	liters	L
ft^3	cubic feet	0.028	cubic meters	m^3
yd^3	cubic yards	0.765	cubic meters	m^3
NOTE: Volumes greater than 1000 l shall be shown in m^3.				
	MASS			
oz	ounces	28.35	grams	g
lb	pounds	0.454	kilograms	kg
T	short tons (2000 lb)	0.907	megagrams (or "metric ton")	Mg (or "t")
	TEMPERATURE (exact)			
°F	Fahrenheit temperature	5(F-32)/9 or (F-32)/1.8	Celcius temperature	°C
	ILLUMINATION			
fc	foot-candles	10.76	lux	lx
fl	foot-Lamberts	3.426	candela/m^2	cd/m^2
	FORCE and PRESSURE or STRESS			
lbf	poundforce	4.45	newtons	N
lbf/in^2	poundforce per square inch	6.89	kilopascals	kPa

APPROXIMATE CONVERSIONS FROM SI UNITS

Symbol	When You Know	Multiply By	To Find	Symbol
	LENGTH			
mm	millimeters	0.039	inches	in
m	meters	3.28	feet	ft
m	meters	1.09	yards	yd
km	kilometers	0.621	miles	mi
	AREA			
mm^2	square millimeters	0.0016	square inches	in^2
m^2	square meters	10.764	square feet	ft^2
m^2	square meters	1.195	square yards	yd^2
ha	hectares	2.47	acres	ac
km^2	square kilometers	0.386	square miles	mi^2
	VOLUME			
mL	milliliters	0.034	fluid ounces	fl oz
L	liters	0.264	gallons	gal
m^3	cubic meters	35.71	cubic feet	ft^3
m^3	cubic meters	1.307	cubic yards	yd^3
	MASS			
g	grams	0.035	ounces	oz
kg	kilograms	2.202	pounds	lb
Mg (or "t")	megagrams (or "metric ton")	1.103	short tons (2000 lb)	T
	TEMPERATURE (exact)			
°C	Celcius temperature	1.8C + 32	Fahrenheit temperature	°F
	ILLUMINATION			
lx	lux	0.0929	foot-candles	fc
cd/m^2	candela/m^2	0.2919	foot-Lamberts	fl
	FORCE and PRESSURE or STRESS			
N	newtons	0.225	poundforce	lbf
kPa	kilopascals	0.145	poundforce per square inch	lbf/in^2

* SI is the symbol for the International System of Units. Appropriate rounding should be made to comply with Section 4 of ASTM E380.

(Revised September 1993)

TABLE OF CONTENTS

Page

EXECUTIVE SUMMARY xi

I. INTRODUCTION 1
1.1 PROCESSES FOR CMA AND KA PRODUCTION FROM WHEY 1
1.2 SCOPE OF STUDY 1

II. BACKGROUND INFORMATION 3
2.1 WHEY AS A BYPRODUCT FROM CHEESE MANUFACTURING 3
2.2 CMA AS A ROAD DEICER 5
2.3 ACETIC ACID AND ACETATE PRODUCTION 8
2.3.1 Aerobic Acetic Acid (Vinegar) Fermentation 8
2.3.2 Anarobic Homoacetogenic Fermentation 9
2.3.3 Anaerobic Digestion 11
2.3.4 Other Bioprocesses 11
2.4 ACETATE PRODUCTION FROM WHEY LACTOSE 11
2.4.1 Homolactic Fermentation of Lactose 12
2.4.2 Homoacetic Fermentation of Lactate 12
2.5 ACETIC ACID RECOVERY AND PURIFICATION 13
2.6 EXTRACTIVE FERMENTATION 15

III. ACETATE PRODUCTION FROM WHEY LACTOSE BY FERMENTATION 19
3.1 INTRODUCTION 19
3.2 CULTURES AND MEDIA 20
3.2.1 Microorganisms 20
3.2.2 Growth Media 20
3.2.3 Fermentation Feedstocks 20
3.3 FIBROUS-BED IMMOBILIZED CELL BIOREACTOR 21
3.3.1 Bioreactor Construction and Operation 21
3.4 CONTINUOUS FERMENTATIONS 22
3.4.1 Homolactic Fermentations 22
3.4.2 Homoacetic Fermentations 23
3.5 FORMULATION OF WHEY MEDIA 24
3.6 CO-CULTURED FERMENTATIONS 26
3.6.1 Batch Fermentations 26
3.6.2 Effects of Lactate 27
3.6.3 Fedbatch Fermentations 29
3.7 ACETATE TOLERANCE 32
3.8 ANAEROBIOSIS 33
3.9 REACTOR LONG-TERM STABILITY 35
3.10 EFFECTS OF CATIONS 35

TABLE OF CONTENTS (continued)

Page

3.11 FERMENTATION SCALE-UP 35
3.11.1 Equipment 35
3.11.2 Reactor Start-Up 37
3.12 HOMOACETIC FERMENTATION OF FRUCTOSE 37
3.13 REACTOR START-UP PERFORMANCE 39
3.14 CONCLUSION AND SUMMARY 40

IV. RECOVERY AND SEPARATION OF ACETIC ACID FROM FERMENTATION BROTH BY EXTRACTION 41
4.1 BATCH EXTRACTION (SHAKE TEST) 41
4.1.1 Solvents 41
4.1.2 Batch Extraction 41
4.2 CONTINUOUS EXTRACTION (PACKED-COLUMN EXTRACTOR) 42
4.2.1 Extraction 42
4.2.2 Solvent Regeneration 43
4.2.3 Continuous Extraction and Back-extraction 44
4.2.4 Effects of Anions on Extraction 47
4.2.5 Gas Stripping for Acetic Acid Recovery 47
4.2.6 Phase Separation 48
4.2.7 Mass Transfer Coefficient 48
4.2.8 Effects of K_D and $K_m a$ on Extraction 48
4.2.9 Effects of V_{org}/V_{aq} on Extraction 48
4.3 BACK-EXTRACTION WITH DOLIME 49
4.3.1 Conclusion and Summary 50
4.4 EXTRACTION USING HOLLOW-FIBER MEMBRANE EXTRACTORS 51
4.4.1 Experimental Set-Up 52
4.4.2 Effects of Flow Rate and pH 53
4.4.3 Effects of Concentration 54
4.4.4 Selectivity 54
4.4.5 Simulated Extractive Fermentation 56
4.4.6 Conclusion and Summary 57

V. CMA PRODUCTION PROCESS AND SAMPLE TESTING 59
5.1 CMA PRODUCTION PROCESS 59
5.2 CMA SAMPLE PREPARATION 60
5.2.1 CMA Samples from Process 1 60
5.2.2 CMA Sample from Glacial Acetic Acid 60
5.2.3 CMA Sample from Process 2 60

TABLE OF CONTENTS (continued)

Page

5.3 CMA SAMPLE ANALYSIS AND TESTING 61
5.3.1 Composition Analysis 61
5.3.2 Deicing Test 61
5.3.3 Comparison to Commercial CMA 63
5.3.4 Conclusion and Summary 63

VI. COST, MATERIALS BALANCE, AND MARKET ANALYSIS 65
6.1 INTRODUCTION 65
6.2 MATERIALS AND ENERGY BALANCE 65
6.2.1 Materials Balance 65
6.2.2 Energy Balance 67
6.3 ECONOMIC AND COST ANALYSES 67
6.3.1 Equipment Costs and Capital Investment 67
6.3.2 Production Costs 69
6.3.3 Return of Investment 71
6.3.4 Effect of Process Scale 71
6.3.5 Liquid CMA 71
6.3.6 Extractive Fermentation Process 72
6.3.7 Effect of Capital Costs 73
6.4 MARKET ANALYSIS 73
6.4.1 Comparison of CMA to Other Deicers 73
6.4.2 CMA Market Survey 74
6.4.2.1 Current Use of CMA in Highway Deicing 74
6.4.2.2 Factors Affecting CMA Use 74
6.4.2.3 Effect of CMA Price on Market Size 74
6.4.3 Market and Supply of Whey 76
6.4.3.1 Other New Uses of Whey 77
6.4.3.2 Supplies of Other Raw Materials 77
6.4.3.3 Other Feedstocks for CMA Production 77
6.5 CONCLUSION AND RECOMMENDATIONS 78
6.5.1 Selection of Plant Site 78
6.5.2 Multiple Products Plant 78

VII. CONCLUSIONS 81

APPENDICES 83
Appendix A. Design of Pilot-Scale Bioreactor for Fermentation 83
Appendix B. Extraction Karr Column 95
Appendix C. Microfiltration System Design 99

BIBLIOGRAPHY 101

LIST OF FIGURES

Figure		Page
1.1	A Fermentation-Extraction Process for CMA Production from Whey Lactose.	2
2.1	A Process Flowsheet Showing Production of WPC and Lactose from Whey.	4
2.2	Corrosion of Steel, Aluminum, and Concrete in Salt, CMA, and Tap Water (Fritzsche, 1992).	7
2.3	Corrosion Performance of Various Chemical Deicers (Fritzsche, 1992).	7
2.4	A Two-Step Extraction Process for Recovering and Concentrating Carboxylic Acids from Dilute Fermentation Broth.	15
3.1	Construction of Convoluted Fibrous Bed and Flow Conditions in the Bed.	21
3.2	Continuous Immobilized Cell Bioreactor with Recirculation and pH Control.	22
3.3	Recycle Batch Immobilized Cell Bioreactor System.	22
3.4	Continuous Homolactic Fermentation of Whey Permeate with Immobilized *S. lactis* Cells at pH 7, 37°C.	23
3.5	Continuous Homolactic Fermentation of Acid Whey with Immobilized *L. helveticus* Cells at pH 5.5, 42°C.	23
3.6	Continuous Homoacetic Fermentation of Lactate Synthetic Medium with Immobilized *C. formicoaceticum* Cells at pH 7.6, 37°C.	24
3.7	Homoacetic Fermentation of Lactate in a Single-Pass Bioreactor without pH Control.	24
3.8	Kinetics of Acetate Production from Lactose By the Co-cultured Fermentation at pH 7.5, 37°C. (A) Whey Permeate Supplemented with 1.5 g/L Casein Hydrolysate, (B) Whey Permeate Supplemented with 2.5 % (v/v) Corn Steep Liquor (40 % T.S.).	26
3.9	Effects of Lactate Formation and Nutrient Concentration on Acetate Production in the Co-cultured Fermentation at pH 7.5, 37°C. (A) WP (27 g/L lactose) Supplemented with 2.5 % (v/v) CSL, (B) WP (41 g/L lactose) Supplemented with 2.5 % (v/v) CSL, (C) WP (50 g/L lactose) Supplemented with 1 % (v/v) CSL.	28
3.10	Kinetics of Fedbatch Fermentations of Whey Permeate Supplemented With 2.5 % (v/v) Corn Steep Liquor at pH 7.5, 37°C. (A) 3.5-L Total Medium Volume, (B) 2-L Total Medium Volume.	30
3.11	Effects of Acetic Acid Concentration on the Specific Growth Rates of the Adapted Cells from the Bioreactor and the Original Culture (B) of *C. formicoaceticum*	33

LIST OF FIGURES (continued)

Figure		Page
3.12	Effects of Acetic Acid Concentration on Acetic Acid Yield, Productivity, and the Specific Growth Rate of the Homoacetogen Grown in the Fibrous-Bed Bioreactor.	34
3.13	Scanning Electron Micrographs of *C. formicoaceticum* (Long Rods) and *L. lactis* (cocci) Co-immobilized on the Fiber Surface in the Fibrous-Bed Bioreactor.	34
3.14	Fedbatch Homoacetic Fermentation with (a) NaOH,(b) $Ca(OH)_2$, and (C) $Mg(OH)_2$ to Control the pH.	36
3.15	Kinetics of Homoacetic Fermentation with Fructose as the Substrate in a Free-Cell System.	37
3.16	Kinetics of Fedbatch Homoacetic Fermentation with Fructose as the substrate:(A) Free-Cell System; (B) Immobilized Cells in the Fibrous-Bed Bioreactor.	38
3.17	Start-Up of 150-L Bioreactor with Fructose Medium to Grow *C. formicoaceticum* in the Fibrous Bed.	39
3.18	Kinetics of Co-cultured Fermentation to Produce Acetate from Whey Lactose in the 150-L Fibrous-Bed Bioreactor.	39
4.1	Performance of Continuous Extraction of Acetic Acid with Alamine 336 in a Packed-Column Extractor.	43
4.2	Comparison of Extraction Performance Between New and Regenerated Solvents.	43
4.3	Experimental Set-Up for Continuous Extraction and Back-Extraction.	44
4.4	Outlet Concentrations of Acetic Acid From Feed and Stripping (Base) Solutions from the Continuous Extraction and Back-Extraction Process.	45
4.5	Performance of Lactic Acid Recovery Using the Two-Step Extraction Process.	45
4.6	Change of Lactate Concentration in the Stripping (Base) Stream with Time.	46
4.7	Lactate Concentration in the Stripping Stream Increased to 70% (w/v).	46
4.8	An Alternative Three-Step Extraction Process for CMA Production.	49
4.9	A Hollow-Fiber Membrane Extraction System for Separating Acetic Acid from the Feed Solution.	52
4.10	Effects of Flow Rate of Aqueous Phase (V_a), Organic Phase (V_o), and Stripping solution (V_s) on the Extraction Rate at pH 5.0 and pH 5.5.	53
4.11	Effects of Amine Content in Solvent and Acid Concentration in the Aqueous Phase on the Extraction Rate.	54
4.12	Selectivity as Affected by the Acetic Acid to Lactic Acid Concentration Ratio and Amine Content in Solvent	55
4.13	Overall Mass Transfer Coefficient as Affected by the Acid Concentration Ratio and Amine Content in Solvent.	55

LIST OF FIGURES (continued)

Figure		Page
4.14	Continuous Extraction of Acetic Acid from Aqueous Solution at pH 5.5 by Adding Lactic Acid (15 % w/v Solution).	56
4.15	A Simulated Extractive Fermentation – Continuous Extraction of Acetic Acid from the Solution Containing 1% Acetic Acid and 0.1% Lactic Acid at pH 5.5 by Continuously Adding the Solution to Maintain a Constant pH 5.5.	57
5.1	Two CMA Production Processes – Process 1 Uses Fermentation to Produce Acetate and Extraction to Recover and Produce CMA; Process 2 Produces a Crude CMA from the Whole Fermentation Broth.	59
5.2	Ice Penetration Performance of Various Deicers at 0°C.	62
6.1	Effect of CMA Price on Projected Market Size: (a) Conservative View; (b) Aggressive View.	75
6.2	A Conceptual Flowsheet for Multiple Fermentation Products from Whey.	79
A1	The 150-liter Fibrous-Bed Bioreactor and Associated Fermentation Pilot Plant Equipment.	84
A2	Schematic Diagram of 150-liter Fibrous-Bed Bioreactor	85
A3	Detailed Engineering Design of 150-liter Fibrous-Bed Bioreactor	86
A4	Engineering Design of Top Plate of 150-liter Fibrous-Bed Bioreactor	87
A5	Engineering Design of Air Distribution of 150-liter Fibrous-Bed Bioreactor	88
A6	Engineering Design Data of 150-liter Fibrous-Bed Bioreactor (a)	89
A7	Engineering Design Data of 150-liter Fibrous-Bed Bioreactor (b)	90
A8	Schematic Diagram of Parameter Control Loops of 150-liter Fibrous-Bed Bioreactor	91
A9	P&I Diagram of 150-liter Fibrous-Bed Bioreactor	92
A10	List of Parts Used in Construction of the Computer Controller and Associated Control-Loop Components for 150-liter Fibrous-Bed Bioreactor	93
A11	Responses of 150-liter Fibrous-Bed Bioreactor to Controlled pH (a) and Temperature (b)	94
B1	A 16-ft Karr Column (2-in Diameter) Used in Extraction Pilot Plant	95
B2	Detailed Schematic of the Karr Column	96
B3	Part List for the Karr Column	97
C1	The Filtration System Used for Sterilization of Whey Permeate	99
C2	Microfiltration System in the Fermentation Pilot Plant	100

LIST OF TABLES

Table		Page
1.1	Major Tasks in Phase I and Phase II Studies.	2
2.1	Characteristics and Compositions of Cheese Whey.	4
2.2	Comparison of Various Products from 100,000 lb Whey Permeate (5 % Lactose).	5
2.3	Environmental Impacts of CMA and Salt (Fritzsche, 1992).	6
2.4	Comparison of Various Fermentation Routes to Produce Acetate from Biomass.	8
2.5	Optimal Growth Conditions and Substrates for Various Anaerobic Homoacetogens.	10
2.6	Acetate Production from Glucose by *Clostridium thermoaceticum*	10
2.7	Typical Distribution Coefficient and pK_a Values for Several Organic Acids.	14
3.1	Growth Test of *C. formicoaceticum* in Whey Permeate with Various Nutrient Supplements.	25
3.2	Fermentations with Various Nutrient Supplements.	27
4.1	Distribution Coefficients for Various Amine Extractants.	42
4.2	Performance of the Two-Step Extraction Process for Organic Acid Recovery.	47
4.3	Results From Back-Extraction of Adogen Extractant with C_aO Slurry.	50
4.4	Results From Back-Extraction of Alamine Extractant with C_aO Slurry.	50
5.1	Compositions of Various CMA Samples.	61
5.2	Comparison of Acetate Content and Ice Penetrating Rate of Various CMA Samples.	62
6.1	Materials/Costs for Production of 24.2 Tons CMA from 1 MM lb Whey Permeate.	66
6.2	Materials and Their Costs for the Production of 7300 Gallons Potassium Acetate (50%) from 0.5 MM lb Whey Permeate.	66
6.3	Energy/Utilities Costs for the Production of 24.2 Tons CMA from 1 MM lb Whey Permeate.	67
6.4	Cost for Major Equipment Items.	68
6.5	Cost Estimation as Percentage of Purchased Equipment.	68
6.6	Estimated Daily Operating Costs for Plants to Produce 24.2 Tons CMA Deicer from 1 MM lb Whey Permeate Per Day.	69
6.7	Estimated Daily Operating Costs for a Plant to Produce 7300 Gallons 50% (w/v) Liquid Potassium Acetate Deicer from 0.5 MM lb Whey Permeate Per Day.	70
6.8	Economics of CMA Production from Whey Permeate.	71
6.9	Effect of Process Scale on CMA Product Cost.	72
6.10	Variations in CMA Product Cost.	72
6.11	Effects of Capital Costs on CMA Product Costs.	73
6.12	Current Situations for Some Dairy Plants Producing Whey.	76

EXECUTIVE SUMMARY

Calcium magnesium acetate (CMA), a mixture of calcium acetate and magnesium acetate, has a deicing ability comparable to salt (NaCl). But unlike salt, CMA is noncorrosive to vehicles, is not harmful to highway concrete and vegetation, and has no significant health or environmental concerns. Currently, the only commercially available CMA deicer is made from glacial acetic acid and dolomitic lime or limestone, and is expensive (~$1000/tonne) as compared with salt and other deicers. Also, a liquid acetate deicer, 50% potassium acetate (KA), is replacing glycols and urea in airport runway deicing. These acetate deicers can be made from cheap feedstocks, such as cheese whey, more economically than the present commercial methods.

The annual production of cheese whey from the U.S. cheese industry is estimated at ~57 billion pounds (26 million metric tonnes). Whey contains about 5% lactose, 1% protein, 1% salts, and 0.1-0.8% lactic acid, and has a high biological oxygen demand (BOD) of ~40,000 mg/L. Currently, only ~50% of the liquid whey produced in the United States is used in human food and animal feed. Disposing of surplus cheese whey is one of the most critical problems facing the dairy industry. Acetate can be efficiently produced from whey (lactose) using an anaerobic mixed culture of homolactic and homoacetic bacteria. To develop and commercialize a process to produce acetate from whey thus would provide a viable solution to the whey disposal problem, meanwhile, providing ~1.7 billion pounds (0.77 million tonnes) per year of low-cost CMA and KA for highway and airport runway deicing from the currently unused whey.

The goal of this project was to develop low-cost acetate deicers from cheap feedstocks, such as cheese whey and other industrial wastes. A novel fibrous-bed bioreactor was developed for fermentation to produce acetic acid from whey lactose by a co-culture of *Lactococcus lactis* and *Clostridium formicoaceticum.* An energy-efficient solvent-extraction process was developed to separate and recover acetic acid from the fermentation broth. Back-extraction or stripping with a base solution to produce acetate salt at a high concentration, >20% (w/v), and simultaneously to regenerate the solvent was also investigated. This project consisted of two phases of work. The technical feasibilities of the proposed fermentation and extraction processes were evaluated at the bench scale in the Phase I study and at the pilot scale in the Phase II study. The economic and market analyses were also conducted to determine the commercial potential of these processes.

The feasibility of using the fibrous-bed bioreactor for long-term, high-rate production of acetate from lactose was first evaluated at the laboratory scale in Phase I. In Phase II, the feasibility of producing a high concentration of acetate broth from whey fermentation with minimal nutrient supplementation, and the scale-up of the fibrous-bed bioreactor were studied. It is concluded that acetate can be produced from whey lactose with a minimal nutrient supplementation with corn steep liquor (CSL) using the fibrous-bed bioreactor. The acetate yield from lactose was 90% or higher, the final acetate concentration from the fermentation was as high as 75 g/L, and the bioreactor had a productivity of 1~2 g/L·h, depending on the acetate concentration. Also, the reactor was stable for long-term operation, either as a continuous reactor or as a recycle batch or fed-batch reactor. These fermentation results indicate that commercial production of acetate from low-cost industrial feedstocks (e.g., whey permeate) and nutrient supplements (e.g., CSL) is

feasible. The pilot scale bioreactor showed a similar kinetic performance to the laboratory bioreactor, indicating that the fermentation technology using fibrous-bed bioreactor should be applicable for production purposes.

A cost-effective method to recover and separate acetic acid from the fermentation broth is needed for economical production of acetate deicers. In Phase I, a two-step extraction process involving extraction with an amine-based extractant and back-extraction with a base solution was investigated using shake tests and a packed-column continuous extractor. The results showed that the process concept is feasible and energy-efficient; however, some difficulties were also identified. The major challenges include: 1) anions present in the fermentation broth would interfere with extraction, and 2) the relatively high pH value in the fermentation broth made extraction inefficient. In Phase II, extraction of acetic acid in a pilot-scale Karr column was studied. In addition, research focused on: 1) understanding the amine extraction chemistry to find methods to overcome the anion interference and pH problems, and 2) developing a hollow-fiber membrane extractor (HFME) for use in extractive fermentation. Methods to enhance extraction rate and selectivity for acetic acid in the acetic/lactic acids mixture system were studied. It is concluded that acetic acid can be effectively extracted and separated from lactic acid using a low content of a secondary amine in a long-chain alcohol. This extraction system also can be used in extractive fermentation to reduce product inhibition problem found in fermentations.

Two alternative methods to produce low-cost acetate deicers from cheese whey were then studied. CMA deicers produced from cheese whey by fermentation and extraction were tested for their acetate content and deicing properties. The CMA solid sample obtained from extraction of the acetic acid present in a dilute aqueous solution and then back-extracted with dolomitic lime to form CMA had about the same acetate content (~70% acetic acid or ~90% CMA) as that of the commercial CMA deicer. The sample from dried whey fermentation broth contained 50% acetic acid or ~63% CMA, with the remaining solids being other organics and salts present in whey. Deicing tests showed that CMA samples from fermentation and extraction had equal or slightly better ice penetration rate than that of the commercial CMA.

Cost analysis showed that CMA can be produced at a product cost of $204-$328/ton, less than 30% of the current market price for commercial CMA, for a plant size of 8400 tons CMA per year. The lower CMA cost should dramatically increase CMA use in the deicing market. Scale-up of these processes is feasible, and the commercial potential of the processes is good.

I. INTRODUCTION

Calcium magnesium acetate (CMA), a mixture of calcium acetate and magnesium acetate, has a deicing ability comparable to salt (NaCl). But unlike salt, CMA is noncorrosive to vehicles, is not harmful to highway concrete and vegetation, and has no significant health or environmental concerns. Currently, the only commercially available CMA deicer is made from glacial acetic acid and dolomitic lime or limestone, and is expensive (~$1000/ton or $1100/tonne) as compared with salt and other deicers. Also, a liquid acetate deicer, 50% potassium acetate (KA) ($4.35/gal or $1.15/liter), is replacing glycols and urea in airport runway deicing. These acetate deicers can be made from cheap feedstocks, such as cheese whey, more economically than the present commercial methods.

The goal of this project was to develop and commercialize a novel fermentation process for acetate (mainly CMA) production from cheese whey, whey permeate, and other lactose-containing waste streams from the dairy industry. It is anticipated that acetate (CMA and KA) production from whey will provide a viable solution to the whey disposal problem. About 1.7 billion pounds (0.77 million tonnes) per year of low-cost CMA and KA can be produced for highway and airport runway deicing from the currently unused whey, provided that an economical production method is available.

1.1 PROCESSES FOR CMA AND KA PRODUCTION FROM WHEY

Acetate can be efficiently produced from whey (lactose) using an anaerobic mixed cultures of homolactic and homoacetic bacteria. The acetate yield from lactose in this fermentation is ~95% weight/weight (wt/wt) and the final acetate concentration in the fermentation broth can be as high as ~75 g/L. The acetic acid-containing fermentation broth can be recovered and further concentrated using a two-step solvent extraction process with extremely low energy consumption. A conceptual process flowsheet for CMA production from whey lactose is illustrated in Figure 1.1. For KA production, a concentrated KOH solution would be used in the back-extraction step to produce 50% weight/volume (wt/v) potassium acetate solution.

1.2 SCOPE OF STUDY

This project consisted of two phases of work. The tasks involved in each phase are summarized in the following table (Table 1.1). The technical feasibility of the proposed process was evaluated at the bench scale in the Phase I study and at the pilot scale in the Phase II study.

In this report, Section II provides background information about CMA, acetate production by fermentation, and acetic acid recovery. The methods used and experimental results obtained from fermentation, extraction, and extractive fermentation studies are detailed in Sections III and IV, respectively. Section V gives descriptions on CMA production processes and sample testing results. Cost, material balance, and market analysis are discussed in Section VI. A bibliography and Appendices are given at the end of this report.

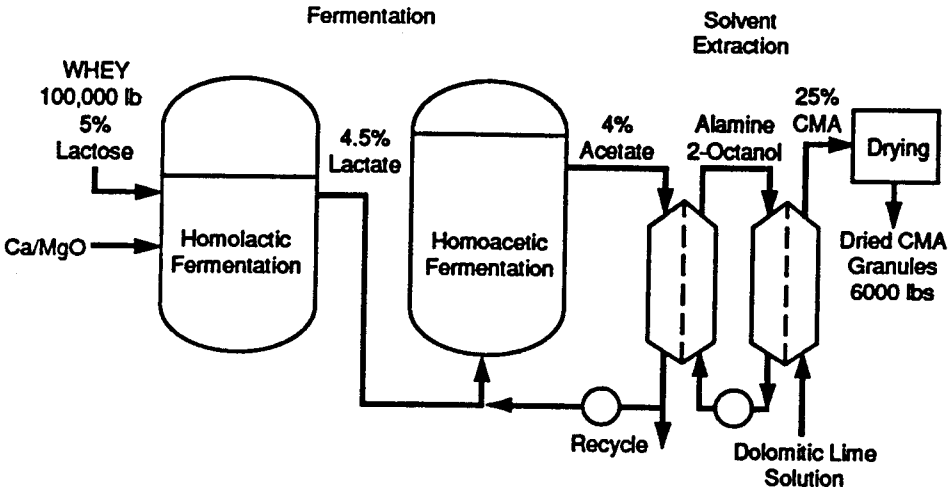


Figure 1.1. A Fermentation-Extraction Process for CMA Production from Whey Lactose.

Table 1.1. Major Tasks in Phase I and Phase II Studies.

Phase I	Activity
Task A	Research and Testing Activities - 1. to evaluate and optimize the fermentation process 2. to evaluate and optimize the extraction process 3. to obtain and test CMA samples for their deicing properties
Task B	Cost and Materials Balance Analysis
Task C	Preliminary Market Analysis and Commercialization Strategy
Task D	Industry Outreach
Task E	Interim Report

Phase II	Activity
Task F	Design for Process Development Unit
Task G	Construct a Process Development Unit
Task H	Optimize the Process Development Unit
Task I	Cost, Material Balance, and Marketing Analysis
Task J	Final Report

II. BACKGROUND INFORMATION

2.1 WHEY AS A BYPRODUCT FROM CHEESE MANUFACTURING

According to the U.S. Department of Agriculture (U.S. Economic Research Service, 1989), U.S. milk production has continuously increased to 147.2 billion pounds (66.9 million tonnes) in 1988. Of that about 89 billion pounds (40.5 million tonnes) of milk was processed to manufacture dairy products, including cheese, butter, ice cream and frozen dairy products; evaporated, condensed, and dried milk; and others. Among these dairies, cheese manufacturing is the largest milk-user, using about 50% of the total processed milk. The annual production of cheese in the United States also has continuously increased to about 6.4 billion pounds (2.9 million tonnes) in 1988. For every pound of cheese manufactured, about 9 pounds (4.1 kilograms) of whey are produced. The estimated yearly production of liquid whey from the U.S. cheese industry is thus more than 57 billion pounds (26 million tonnes).

Whey, as a byproduct from the manufacture of cheese and casein, contains about 5% lactose, 1% protein, 1% salts, and 0.1-0.8% lactic acid. The biological oxygen demand (BOD) of whey is thus high, ~40,000 mg/L. Currently, only ~50% of the liquid whey produced in the United States is used in human food and animal feed. A new use must be found for the remaining surplus whey or it must be treated as a pollutant because of the high BOD of whey. Given the continuous increase in milk and cheese production in the United States and throughout the world, disposing of surplus cheese whey is one of the most critical problems facing the dairy industry (Yang and Silva, 1995).

Depending on the cheese-making process, there are two principle types of whey: sweet whey and acid whey (Harper and Hall, 1976). Table 2.1 shows the compositions of various whey products. Most of whey and modified whey products are made from sweet whey. The largest use of whey is dried whey powder as a food ingredient or animal feed. However, the conversion of sweet whey into a food-grade ingredient or animal feed by evaporation and drying is energy intensive and is only economical for large plants. The market for dried whey powder is also unstable and very competitive. Small to medium-size plants (less than 10 million lb annually) cannot justify the costs for producing dried whey powder. Also, only 8.2% of the total acid whey generated in the United States becomes a marketable product. For these cheese plants, whey is usually disposed of by either landspreading or discharging it into a municipal waste treatment system. However, landspreading was recently banned in several states, including New York.

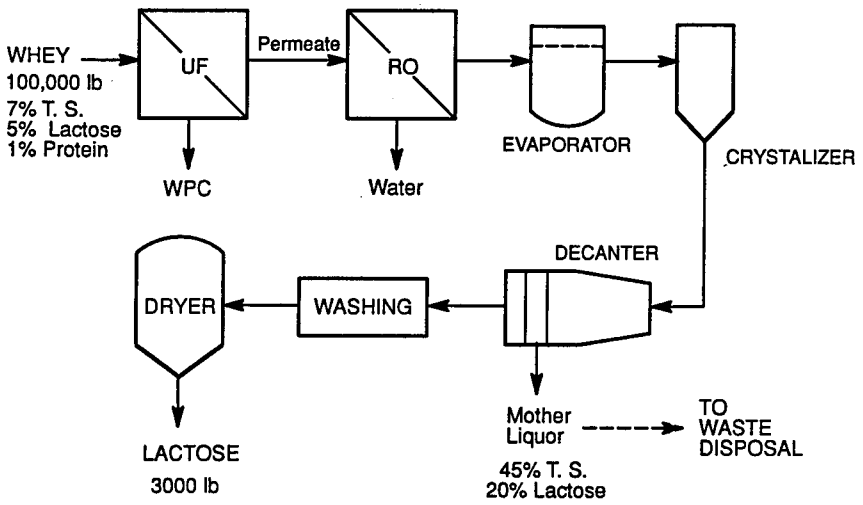
For many large dairy plants, whey proteins, which make up 15% to 22% of the total milk proteins, are recovered and concentrated by ultrafiltration (UF). The product, whey protein concentrate (WPC), has good market value because of its excellent functional properties and nutritional values. However, the remaining lactose stream (whey permeate) from ultrafiltration generally has no use and causes disposal problems for many dairies.

Lactose accounts for 70%-80% of total whey solids. It can be readily isolated and purified from whey permeate by crystallization (Figure 2.1). However, the U.S. and world markets for lactose are cyclical and often very competitive. The market prices for lactose have fluctuated between $0.10/lb ($0.22/kg) and $0.40/lb ($0.88/kg) in the recent past. Furthermore, the lactose recovery yield from

whey permeate is low (only about 60%), and the waste stream (mother liquor) from the crystallization process contains high salts (>20%), high lactose (~20%), and high BOD. Because of the high salt content, this mother liquor has limited applications and generally requires costly treatment. The weak marketing potential for lactose and the increasing waste treatment cost have prompted a continuous search for better uses of whey permeate.

Table 2.1. Characteristics and Compositions of Cheese Whey.

Composition (%)	Sweet whey		Acid whey	
	Fluid	Dried	Fluid	Dried
Moisture	93.7	3.5	93.5	4.0
Total solids	6.35	96.5	6.5	96.0
Lactose	4.85	75.0	4.9	67.4
Protein	0.8	13.4	0.75	12.5
Fat	0.5	0.8	0.04	0.6
Lactic acid	0.05	0.2	0.4	4.2
Ash	0.5	7.3	0.8	11.8
P		0.7		1.1
Ca		0.7		1.9
K		2.1		2.3
Na		1.3		1.1
pH value	~ 5.5		4.0 – 4.5	



2.2 lb = 1 kg

Figure 2.1. A Process Flowsheet Showing Production of WPC and Lactose from Whey. UF - ultrafiltration. RO - reverse osmosis. T.S. - total solids.

The utilization of whey lactose as a fermentation feedstock has long been of interest to the dairy industry (Hobman, 1984). A wide range of products can be obtained from whey fermentations, including single cell protein, methane, alcohols (ethanol, butanol), organic acids (lactic, acetic, propionic, citric), vitamins, and biopolymers such as xanthan gum (Yang and Silva, 1995). However, production of a suitable fermentation product from whey (permeate) must consider technological, market, and economic factors. Presently, none of the existing whey fermentation processes have wide-scale use in industry (Yang and Silva, 1995). Table 2.2 compares a number of products that can be made from whey permeate.

Table 2.2. Comparison of Various Products from 100,000 lb Whey Permeate (5% Lactose).

Product	Quantity	Unit Price	Total Value	Use	Market Size
Lactose	3000 lb	$0.20/lb	$ 600	Food	200 MM lb
Methane	25,000 ft^3	$5/1000 ft^3	$ 125	Energy	On-site use
Ethanol	320 gal	$1.5/gal	$ 480	fuel	~1 billion gal
Acetic acid	4,500 lb	$0.38/lb	$1700	chemical	~2 MM ton
CMA	6000 lb	$0.5/lb	$ 3000	deicer	~10,000 ton
K-Acetate, 50%	1500 gal	$4.0/gal	$ 6000	deicer	~5 MM gal
Lactic acid	4500 lb	$1.0/lb	$ 4500	food, polylactides	~60 MM lb potentially big
Propionic acid	3000 lb	$0.46	$ 1400	chemical	~400 MM lb
Ca-propionate	3000 lb	$1.5/lb	$ 4500	food	small
Xanthan gum	3500 lb	$5.0/lb	$17500	food, oil recovery	~70 MM lb

2.2 lb = 1 kg; 2200 lb = 1 tonne; 0.26 gal = 1 liter.

2.2 CMA AS A ROAD DEICER

From 10 to 14 million tons (9.1 to 12.7 million tonnes) of road salt are used annually in the United States and Canada. Salt is an extremely effective snow and ice control agent and is relatively inexpensive. However, a recent study in New York State showed that, while a ton of road salt costs only $25, it causes more than $1400 in damage (Hudson, 1987). Salt is corrosive to concrete and metals used in the nation's infrastructure. Salt also is harmful to vegetation and poses serious threats to environment and groundwater quality in some regions (Fritzsche, 1992). The Federal Highway Administration has long recognized this problem and has identified CMA as one of the most promising alternative road deicers (Chollar, 1984). Consequently, there have been numerous studies concerning the effects of CMA on the environment (Fritzsche, 1992; Hiatt et al., 1988; Slick, 1988). The environmental impacts from salt and CMA are compared in Table 2.3.

CMA is a mixture of calcium acetate and magnesium acetate. It is currently being manufactured by reacting glacial acetic acid with dolomitic lime (Ca/MgO) or limestone ($Ca/MgCO_3$). CMA has a deicing ability comparable to salt, but is noncorrosive and harmless to vehicles, highway concrete, bridges, and vegetation. It is biodegradable and has no identified environmental concerns (Fritzsche, 1992). However, CMA's present cost of ~$1000/ton ($1100/tonne), versus ~$30/ton ($33/tonne) for salt, makes it too expensive for widespread use, even though some studies have

shown that all of its material cost can be offset by the savings in infrastructure replacement costs. Consequently, CMA is currently used only in limited areas where corrosion control is required or in environmentally sensitive areas to protect vegetation and ground water from salt poisoning (Harrach and Wyatt, 1990). Using CMA as a deicer, however, will be cost-effective and better accepted if its price can be reduced to $300-$400/ton ($330-$440/tonne) (Ministry of Transportation of Ontario, 1989). It is thus important to produce low-cost CMA deicers from alternative feedstocks such as biomass and industrial wastes (Bryan, 1992; Compere and Griffith, 1975; Ljungdahl, 1983; Yang, 1991; Yang et al., 1997).

Table 2.3. Environmental Impacts of CMA and Salt (Fritzsche, 1992).

Environmental Impact, Calcium Magnesium Acetate versus Road Salt		
Environmental Impact	**Calcium Magnesium Acetate, $CaMg_2(C_2H_3O_2)_2$**	**Road Salt, NaCl**
Soils	Biodegradable in soil. No adverse effect on soil compaction and strength. Increases soil permeability.	May accumulate in soil. Breaks down soil structure, increases erosion. Causes soil compaction, which decreases permeability.
Vegetation	Little or no adverse effect. May stimulate roadside plant growth. Acetate ion is the most abundant organic acid metabolite found in nature.	Osmotic stress and soil compaction harm root systems. Spray causes foliage dehydration damage. Many plant species are salt sensitive.
Groundwater	Poor mobility in soil, unlikely to reach groundwater. Ca, Mg increase water hardness.	Mobile Na and Cl ions readily reach groundwater. Na and Cl in well water increase alkalinity and hardness.
Surface water	Potential for oxygen depletion through BOD at concentrations greater than 100 mg/L in closed systems. Decomposes in 5 days at 20°C, 10 days at 10°C, 100 days at 2°C. Will not stimulate algae growth.	Causes density stratification in ponds and lakes, which can prevent reoxygenation. Increases runoff of heavy metals and nutrients through increased erosion.
Aquatic life	Less toxic to trout than salt. Minimal effect on trout eggs up to 5 times expected max runoff concentration of 1000 mg/L. No effect on food chain (zooplankton, daphnia, bluegill, and fathead minnows) up to 1000 mg/L.	Monovalent Na, Cl ions stress osmotic balance. Toxic levels: Na, 500 mg/L stickleback; Cl, 400 mg/L trout.
Human/mammalian	Mild skin and eye irritant. Vinegar odor. Acute oral LD50 in rats greater than 5000 mg/kg. Essentially non-toxic.	Sodium linked to heart disease, hypertension. Cl causes unpleasant taste in drinking water. Mild skin and eye irritant. Acute oral LD50 in rats approx. 3000 mg/kg. Slightly toxic. Contributes to winter road wildlife kills.
Water treatment plants	No significant increase in chemical oxygen demand or impact on bacterial activity.	No significant impact at expected concentrations.
Air pollution	Can reduce sand use and resulting particulate emissions.	Can reduce sand use and resulting particulate emissions.

Detailed discussions of the comparison between salt and CMA for highway deicing can be found in a recent report from the Transportation Research Board of National Research Council (1991) and in the book *Chemical Deicers and the Environment* (D'Itri, 1992). Figure 2.2 shows the corrosion rates of salt and CMA to steel and concrete. Figure 2.3 compares the corrosion performance of various chemical deicers. As shown in these figures, CMA is not only non-corrosive, but it also inhibits corrosion. CMA can thus be used in mixtures with salt to reduce corrosion, and also reduces the deicing costs as well as the corrosion damage in salt-insensitive environments.

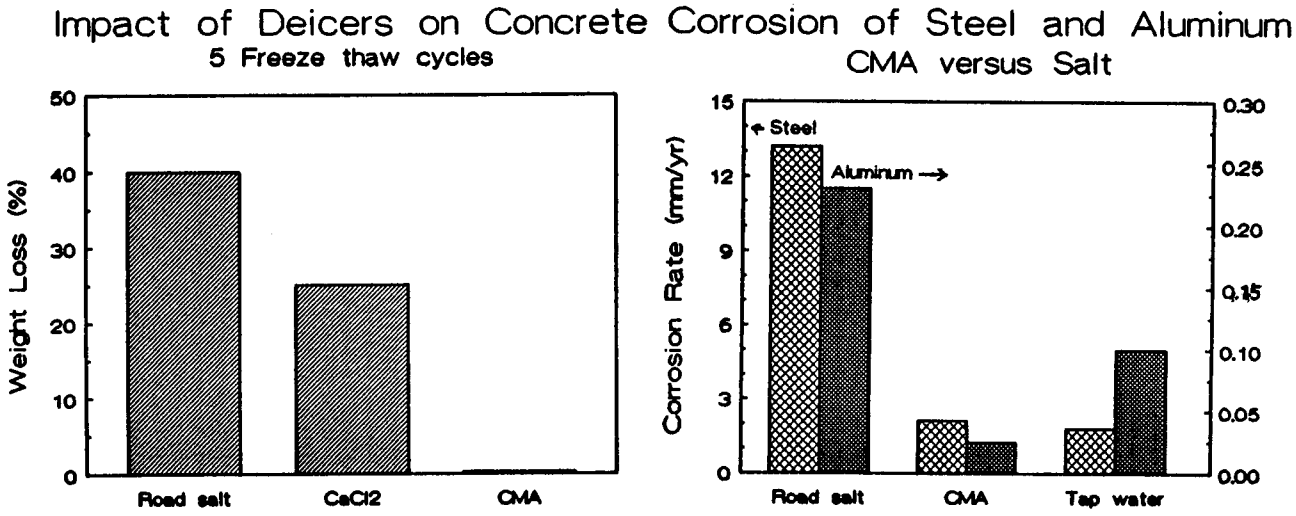


Figure 2.2. Corrosion of Steel, Aluminum, and Concrete in Salt, CMA, and Tap Water.

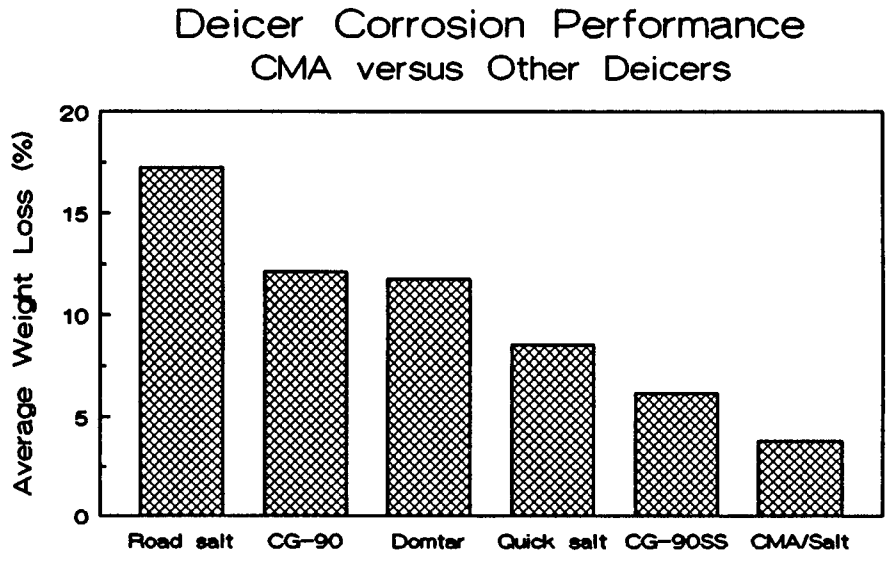


Figure 2.3. Corrosion Performance of Various Chemical Deicers (Fritzsche, 1992).

2.3 ACETIC ACID AND ACETATE PRODUCTION

Acetic acid is an important raw material in the chemical industry. In the past several years, the yearly production of acetic acid in the United States has continuously increased to ~4.68 billion pounds (2.13 million tonnes) in 1995. At present time, commercial production of glacial acetic acid is exclusively by petrochemical routes and costs ~$0.32/lb (~$0.70/kg). Various amounts of acetic acids also may be recovered from various industrial waste streams; however, the recovery of acetic acid from industrial waste streams has not been economically viable due to limited markets for the recycled acetic acid. Only very recently has the development effort to recover acetic acid by solvent extraction received some industry attention.

Several different fermentation routes have been widely studied for their potential use to produce acetic acid or acetate from biomass. The characteristics of these fermentation methods are summarized in Table 2.4 and discussed in detail in the following sections.

Table 2.4. Comparison of Various Fermentation Routes to Produce Acetate from Biomass.

	Aerobic Vinegar Fermentation[1]	Anaerobic Homoacetic Fermentation		Anaerobic Digestion[3]
		C. thermoaceticum	*C. formicoaceticum*[2]	
Substrate	Glucose/Ethanol	Glucose	Lactate	Cellulosics
Acetate yield	<60%	>80%	>95%	30% - 80%
Acetate conc. (w/v)	6% - 10%	2% - 10%	3% - 7.5%	<3%
Fermentation time	1 - 3 days	1 - 7 days	2 - 7 days	6 - 15 days
Energy requirement	high in fermentation	medium	low	high in product recovery

[1]requires ethanol fermentation of glucose first; ethanol is the substrate for acetic acid fermentation.
[2]lactate can be obtained from carbohydrates through homolactic acid fermentation.
[3]methanogenic activities are suppressed to allow acetate accumulation.

2.3.1. Aerobic Acetic Acid (Vinegar) Fermentation

Acetic acid (vinegar) traditionally has been produced from ethanol derived from sugar fermentation. This process generally involves two steps (Eq. 2.1): (1) fermentation of sugar to ethanol by yeasts such as *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*, and (2) oxidation of ethanol to acetic acid by species of *Acetobacter* (Ebner, 1981).

$$C_6H_{12}O_6 \rightarrow 2CO_2 \uparrow +2C_2H_5OH \xrightarrow{O_2} 2CH_3COOH \qquad (2.1)$$

This process has two major disadvantages: (1) The acetic acid yield is, at most, only two-thirds of the sugar source, since up to one-third of the organic carbon is lost as CO_2 during the ethanol fermentation. In practice, the yields of ethanol and acetic acid are rarely more than 90% and 85% of the theoretical value, respectively (Ebner and Follmann, 1983). With an overall efficiency of 75%, only 0.5 pounds (0.227 kg) of acetic acid can be produced per pound (0.454 kg) of sugar used;

(2) strict aeration is required for the growth of *Acetobacter* to convert ethanol to acetic acid. Improper aeration may cause serious damage to the acetic acid bacteria, or result in overoxidation of acetic acid to CO_2 and water (Ebner, 1981). As a result, the conventional aerobic vinegar fermentation process suffers from low yield and high production cost, and thus cannot compete well with the natural gas-based synthetic process (Ghose and Bhadra, 1985).

Earlier studies conducted by Stanford Research Institute (SRI) concluded that CMA production via aerobic vinegar fermentation of glucose or hydrolyzed corn starch was not economically competitive to warrant further consideration (Marynowski et al., 1984). This prompted the search of anaerobic homoacetogens for acetic acid or CMA production.

2.3.2 Anaerobic Homoacetogenic Fermentation

Recently, there has been increasing interest in producing acetic acid from renewable resources using anaerobic bacteria (Detroy, 1981; Huang et al., 1998; Huang and Yang, 1998; Ljungdahl et al., 1986; Marynowski et al., 1984; Yang et al., 1993; Yang and Chollar, 1996). In contrast to the aerobic vinegar process, nearly 100% of the substrate carbon can be recovered in the product, acetic acid, by anaerobic fermentation (Eq. 2.2).

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad (2.2)$$

The actual acetic acid yield from glucose is usually greater than 80% (wt/wt). However, the final concentration of acetic acid produced from an anaerobic process is usually very low, only about 2% (Schwartz and Keller, 1982a), compared with ~10% obtained from the aerobic process (Ebner and Follmann, 1983; Park et al., 1991a, b). This low product concentration leads to a prohibitory, high energy requirement for the recovery of the acetic acid produced in the anaerobic process (Ghose and Bhadra, 1985), though the overall energy consumption is still lower than that for the aerobic process.

Many anaerobic bacteria can convert carbohydrates to acetic acid via homofermentation (Table 2.5). However, *Clostridium thermoaceticum* is the only one that has been extensively studied for its ability to convert glucose, xylose, and cellulose to acetic acid at 60°C (Parekh and Cheryan, 1990a, b; Schwartz and Keller, 1982a, b; Wang and Wang, 1984; Wang et al., 1978). Recently, Parekh and Cheryan (1994a) reported a mutant strain of *C. thermoaceticum* that was able to produce acetate concentration as high as 10% (w/v) in a fedbatch fermentation with cell recycle. The reactor productivity was ~0.8 g/(L·h) and the acetate yield from glucose was ~80%. However, the results were obtained with a synthetic medium with a high yeast extract content. Efforts to use inexpensive industrial feedstocks for acetate production using this strain have not been as successful. Table 2.6 summarizes some prior results obtained from this microorganism.

Other known anaerobic homoacetogens include *C. formicoaceticum* (Andreesen et al., 1970), *C. aceticum* (Braun et al., 1981), *C. thermoautotrophicum* (Wiegel et al., 1981), *C. magnum* (Schink, 1984), *Acetobacterium woodii* (Balch et al., 1977), *A. carbinolicum* (Eichler and Schink, 1984), and *Acetogenium kivui* (Leigh et al., 1981). Ljungdahl et al. (1985) studied three thermophilic

acetogens - *C. thermoaceticum*, *C. thermoautotrophicum*, and *A. kivui* - for their abilities to produce CMA from glucose. It was concluded that although *C. thermoaceticum* was the most desirable, none would have successful industrial applications due to the high cost of the feedstock (glucose) used in the fermentation.

Table 2.5. Optimal Growth Conditions and Substrates for Various Anaerobic Homoacetogens.

Organism	Temp	pH	Growth Substrate
Clostridium			
Aceticum	30°C	8.3	Fructose, Pyruvate, CO_2/H_2, CO/H_2
formicoaceticum	37°C	7.6	Fructose, Pyruvate, Pectin, Lactate, Galacturonate
thermoaceticum	60°C	7.0	Fructose, Pyruvate, Glucose, Xylose, (Lactate)*
thermoautotrophicum	60°C	5.7	Fructose, Glucose, Galactose, Glycerate, Methanol, Formate, CO_2/H_2, CO/H_2, (Lactate)*
magnum	30°C	7.0	Fructose, Glucose, Sucrose, Xylose, Citrate, Malate
Acetobacterium			
Woodii	30°C	6.7	Fructose, Glycerate, Glucose, Lactate, CO_2/H_2, Formate
Carbinolicum	27°C	7.0	Fructose, Glucose, Pyruvate, Lactate, Formate, Aliphatic alcohols C_1-C_5, CO_2/H_2
Acetogenium kivui	66°C	6.4	Fructose, Pyruvate, Formate, Mannose, Glucose, CO/H_2

*only a few strains can utilize lactate for growth.

Table 2.6. Acetate Production from Glucose by *Clostridium thermoaceticum*.

Strain	Glucose (g/L)	Acetate (g/L)	Acetate Yield[1] (g/g)	Fermentation time (h)	Reference
ATCC 39073	20	14.4	0.91	-	Ljungdahl et al., 1985
S-3	21	20.0	0.98	180	Schwartz & Keller, 1982a
Wood	20	15.0	0.83	-	Schwartz & Keller, 1982b
DSM-521	40	37.0	0.92	130	Wang et al., 1978
DSM-521	40	30.	0.85	28	Wang et al., 1978
ATCC 39289	19	15.4	0.93	120	Parekh and Cheryan, 1990a
ATCC 39289*	35	29	0.83	140	Parekh and Cheryan, 1990b
ATCC 39289*	40+	~100	0.8	150	Parekh and Cheryan, 1994a

[1]Yield is based on glucose utilization.
*Mutant strain adapted to high-acetate environment.
+Fedbatch fermentation.

None of the homoacetogenic bacteria can ferment lactose; however, several can readily convert lactic acid to equal amounts of acetic acid. Since homolactic fermentation has been widely applied in the dairy industry for producing lactic acid from lactose, it is feasible to produce acetic acid from

whey permeate by converting lactose to lactic acid, and then to acetic acid using homolactic and homoacetic bacteria, respectively (Tang et al., 1988). Also, lactic acid is abundant in many industrial waste streams, such as corn steep liquor from the corn wet milling industry.

2.3.3 Anaerobic Digestion

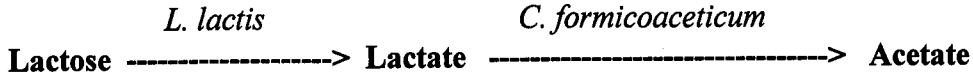
Anaerobic digestion of biomass as a means of producing CMA in a mixture of organic-acid salts has also been studied by Wise and his coworkers. In their processes, growth of methanogens was suppressed to allow acetate accumulation. Sewage sludge (Trantolo et al., 1990; 1991), woody biomass (DeSouza and Wise, 1991; Wise and Augenstein, 1988), and in principle, any low-grade biomass such as cheese whey (Dynatech, 1988) can be used in this process. However, the reaction rate is extremely low and acetate yield is only 30% ~ 80%, depending on the fermentation condition. The acetate concentration obtained from this process was also very low, only 0.8%, although theoretically 3% is possible. Other organic acids present in the product stream include, mainly, propionic and butyric acids. The major problem of using this process is that the reactor performance is not stable because many undefined mixed cultures are involved and are difficult to control.

2.3.4. Other Bioprocesses

As noted in Table 2.5, some homoacetogens may use hydrogen gas and single-carbon compounds, such as CO, CO_2, methanol, and formate, to produce acetic acid. This provides a biological method to convert syn-gas (CO, CO_2, and H_2) to acetic acid, though the low solubilities and large volume associated with the gaseous feedstocks might limit their industrial applications. BioEngineering Resources Inc. (Fayetteville, AK) is currently developing a process to produce acetic acid from biomass and industrial wastes through gasification followed by fermentation of the syn-gas. There are large quantities of waste gas generated from power plants and they could be inexpensive feedstock sources for this process; however, collection of the waste gas is difficult and could render the process uneconomical.

2.4 ACETATE PRODUCTION FROM WHEY LACTOSE

As discussed earlier, neither anaerobic digestion nor aerobic vinegar process is attractive for CMA production. There is no homoacetogen which can directly ferment lactose to acetate, either. However, acetate can be produced efficiently from lactose via two anaerobic fermentation processes. The first one is to use propionic bacteria to ferment lactose to propionate and acetate (Lewis and Yang, 1992). Acetate, however, is not the major product (but it may be economically recovered as a byproduct) from this process. The second process, which was developed in this study, is to use a co-culture consisting of homolactic and homoacetic bacteria. These two bacteria sequentially convert lactose to lactate and then to acetate, with acetate yield from lactose greater than 90% (Yang et al., 1991; 1992).



2.4.1. Homolactic Fermentation of Lactose

Lactic acid bacteria are routinely used in cheese manufacturing. Some food-grade lactic acid and sodium lactate are made from sugar fermentations by using homolactic bacteria. Recently, there has been increasing interest to produce polymer-grade lactic acid from biomass via fermentations. Lactic bacteria are generally referred to as a group of gram-positive rods (*Lactobacteriaceae*) and cocci (*Streptococcaceae*) that ferment carbohydrates to lactic acid solely (homofermentative), or to lactic and acetic acids, ethanol, and carbon dioxide (heterofermentative). The organisms used for the industrial production of lactic acid are homofermentative. In general, *L. delbrueckii* is used to ferment glucose, maltose, or dextrose, and *L. bulgaricus* or *L. lactis* (formerly named *S. lactis*) is used to ferment lactose. More recently, *L. helveticus* was also used to produce lactic acid from whey permeate in a continuous process (Aeschlimann et al., 1990). There are also other species that may work well with whey.

2.4.2 Homoacetic Fermentation of Lactate

Among all the homoacetogens, only *A. woodii*, *A. carbinolicum*, and *C. formicoaceticum* can ferment lactate to acetate. *A. woodii* can grow at a temperature below 32°C and a pH between 4.5 and 6.8 (Balch et al., 1977). *C. formicoaceticum* can grow at a temperature around 35°C and a pH between 6.6 and 9.6 (Yang et al., 1988). Both organisms are inhibited by acetic acid, but *C. formicoaceticum* can tolerate a higher concentration as compared with *A. woodii* (Yang, 1984). *C. formicoaceticum* is also more active than *A. woodii* when grown on lactate at neutral pH and mesophilic temperatures (Yang, 1984). *A. carbinolicum* is a new isolate similar to *A. woodii*. Thus, *C. formicoaceticum* would be the most appropriate one to convert lactate to acetate. However, the best homoacetogen for CMA production will be dependent on fermentation conditions (especially pH) and process design.

The conversion of lactate to acetate by homoacetogen (e.g., *C. formicoaceticum*) is relatively slow and is the rate-limiting step for the co-cultured fermentation to produce acetate from lactose. *C. formicoaceticum* is a gram-negative, strictly anaerobic, mesophilic bacterium (Yang et al., 1987). It homofermentatively converts lactate to acetate at mesophilic temperatures (30 to 42°C) and at pHs between 6.6 and 9.6 (Yang et al., 1987). This bacterium grows at an optimal pH of 7.6 and an optimal temperature of 37°C. Acetate formation from lactate was found to be growth-associated; i.e., it happened simultaneously with cell growth. Approximately 0.98 g of acetic acid was formed from each gram of lactic acid consumed. Although the fermentation was strongly inhibited by acetic acid, cell growth was observed at acetate concentrations of up to ~5% (0.8M) in batch fermentations of lactate with hydroxide being used to maintain the reactor pH at ~7.6 (Tang et al., 1989).

The kinetics and effects of pH and acetic acid on the growth of *C. formicoaceticum* have been reported elsewhere (Tang et al., 1989). A mathematical model for this fermentation at various pH values has also been developed and can be used for reactor design and simulation (Yang et al., 1988).

2.5 ACETIC ACID RECOVERY AND PURIFICATION

Fermentatively produced acetic acid usually is recovered by solvent extraction and/or azeotropic dehydration. However, the acetic acid produced in the anaerobic fermentation at pH ~7 is in the form of acetate salt. Conventional solvents can extract only free acid from the fermentation broth (Kertes and King, 1986). Previous attempts to overcome this problem by adapting the microbes to the acidic pH (Schwartz and Keller, 1982a, b) or by acidifying the broth before extraction failed to reduce the production costs to a competitive level (Busche et al., 1982).

The highest concentration of acetic acid that most anaerobic homoacetogens can tolerate is lower than 4%. However, conventional extraction solvents require a concentration of acetic acid higher than 10% and, therefore, an evaporation process is usually recommended prior to solvent extraction (Ghose and Bhadra, 1985). Nevertheless, if a highly efficient extractant is available, the heating process can be reduced to a level sufficient only for killing the microbes in the liquor, if it is necessary. Solvents with a high distribution coefficient can be used to extract acetic acid from a low concentration solution (Helsel, 1977). These include trioctylphosphine oxide (TOPO), and long-chain aliphatic amines.

Recently, extractive recovery of carboxylic acids, e.g., acetic acid, from dilute, aqueous solutions, such as fermentation broth and wastewater, which have acid concentrations lower than 10%, has received increasing attention (Helsel, 1977; Jagirdar and Sharma, 1980; Busche et al., 1982; Robinson and Cha, 1985; Kertes and King, 1986; Tamada et al., 1990; Faharim et al., 1992; Starr and King, 1992). Organic solvents used for extraction can be categorized into three major types: 1) conventional oxygen-bearing and hydrocarbon extractants, 2) phosphorus-bonded oxygen-bearing extractants, and 3) high-molecular weight aliphatic amines (Kertes and King, 1986). Solvent extraction with conventional solvents such as alcohols, ketones, ethers, and aliphatic hydrocarbons is not efficient when applied to dilute, carboxylic acid solutions because of the high aqueous activity of carboxylic acids resulting in low distribution coefficients.

However, carboxylic acid extractions with organophosphates, such as trioctylphosphine oxide (TOPO) and tri-n-butyl phosphate (TBP), and aliphatic amines have large distribution coefficients. Aliphatic amines are slightly more effective and less expensive than phosphorus-bonded oxygen-bearing extractants (Wardell and King, 1978). Several aliphatic amines have been used successfully to extract carboxylic acids (Wardell and King, 1978; Ricker et al., 1979; Wennersten, 1983; Kertes and King, 1986; Tamada and King, 1990; Yabannavar and Wang, 1991a; Eyal and Canari, 1995). The strong amine interactions with the acid allow for the formation of acid-amine complexes, and thus provide for high distribution coefficients. In addition, the high affinity of the organic base for the acid gives selectivity for the acid over non-acidic components in the mixture. However, primary amines are too soluble in water to be used with aqueous solutions. Secondary amines may be subject to amide formation upon regeneration by distillation. Consequently, long-chain tertiary amines have received the most attention. Extraction of acetic acid with various solvents for acetate production has been recently reviewed (Althouse and Tavlarides, 1992).

We have studied the extraction of organic acids using aliphatic amines (Yang et al., 1991). Different types of amine extractants, effects of solution pH, and effects of diluent have been studied

with several organic acids. The extraction coefficient, K_D, was found to be greatly affected by the solution pH. In general, the K_D value increases with a decrease in the pH value except at extremely high or low pH where K_D does not change with the pH. The pH effect on distribution coefficient, K_D, can be modeled by the following equation (Eq. 2.3):

$$K_D = \frac{K_1 + K_2 K_a/[H^+]}{1 + K_a/[H^+]} \tag{2.3}$$

where K_a is the equilibrium or dissociation constant of the acid, and K_1 and K_2 are two intrinsic distribution coefficients at extremely low and high pH values, respectively. At low pH values or $[H^+] >> K_a$, $K_D = K_1$. At high pH values or $[H^+] << K_a$, $K_D = K_2$ (which is zero for most amine extractants, except for Aliquat 336). The typical K_1 values and K_a for several organic acids of interest are listed in Table 2.7. It is clear that the amine extractants have higher distribution coefficients for longer-chain carboxylic acids. Thus, it is possible to separate the organic acid mixture using this solvent extraction method. For example, propionic acid can be separated and purified from propionic acid fermentation broth containing propionic and acetic acid. The addition of a polar diluent (e.g., 2-octanol) to the amine extractant also greatly increases the K_D value of the extractant (Yang et al., 1991).

Table 2.7. Typical Distribution Coefficient and pK_a Values for Several Organic Acids.

Organic Acid	Distribution Coeff., K_1		$pK_{a,25°C}$
	Alamine 336	Adogen 283	
Acetic acid	2.8	16.1	4.76
Lactic acid	3.5	8.4	3.86
Propionic acid	8.4	~20	4.88
Butyric acid	16.5	~50	4.76

Since Alamine 336 and Adogen 283 only extract the undissociated acid and will not extract acids under basic conditions, they can be easily regenerated by back-extraction with an alkaline solution. This is the principle that we used in designing the two-step extraction process shown in Figure 2.4, to produce concentrated organic salts from a dilute acid solution. In this process, a solvent with a high distribution coefficient can be used to extract organic acids from a fermentation broth with a pH value below 6 (preferably at 3). Back-extraction with an alkaline solution (with pH above 10) is then followed to regenerate the extractant and to form an organic salt in a concentrated solution. This two-step extraction method would provide an energy-efficient way to recover, separate, and concentrate organic acids from a dilute fermentation broth. The feasibility of such a process for CMA production from acetic acid has been demonstrated in a recent study (Reisinger and King, 1995), but further work is necessary before the process can be developed for industrial use.

Separation of acetic acid by adsorption with ion exchange resins is not feasible at the present time. Steam or gas stripping of volatile compounds, such as acetic acid, in a dilute solution is not

economically feasible, either. An energy-efficient steam stripper with caustic solution to improve stripping efficiency has been described recently (U.S. Patent 4,917,769, 1990). However, due to the low relative volatility of acetic acid to water (~0.69), a large number of trays or equilibrium stages are required to obtain 90% recovery. Also, the tray design in this stripping tower is quite complicated. It is difficult, if possible, to use high concentrations of lime slurry as the caustic in this stripper.

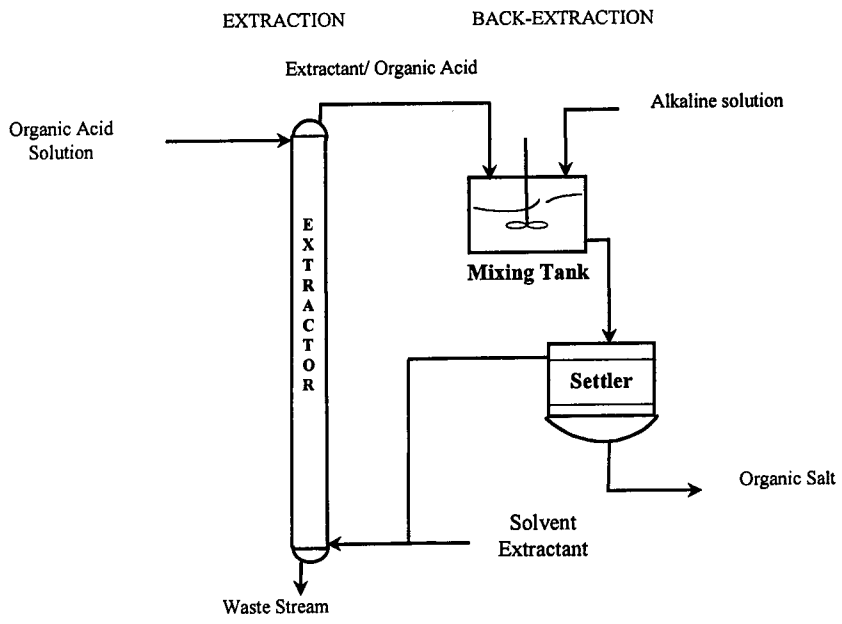


Figure 2.4. A Two-Step Extraction Process for Recovering and Concentrating Carboxylic Acids From Dilute Fermentation Broth.

2.6 EXTRACTIVE FERMENTATION

Extractive fermentation is a relatively new technology that has not yet been used in industry (Bar and Gainer, 1987; Daugulis, 1988; Honda et al., 1987; Weilnhammer and Blass, 1994). Extractive fermentation has been extensively studied for alcohol fermentation (Roffler et al., 1984). More recently, extractive fermentation with organic solvents or adsorbents and other types of integrated fermentation-separation processes, such as electrodialysis fermentation, have also been studied for several organic acid fermentations (Chen and Lee, 1997; Evans and Wang, 1990; Hatzinibolaou and Wang, 1992; Honda et al., 1995; Jin and Yang, 1998; Katzbauer et al., 1995; Kwon et al., 1996; Lewis and Yang, 1992; Normura et al., 1988; Planas et al., 1996; Scholler et al., 1993; Seevaratnam et al., 1991; Solichien et al., 1995; Srivastava et al., 1992; Wang et al., 1994; Wang and Zhao, 1995; Yabannavar and Wang, 1991b). However, very little has been done with anaerobic homoacetic acid fermentation (San Nicolas et al.,1997; Yang, 1988).

The advantages for extractive fermentations include increased reactor productivity, ease in reactor pH control without requiring base addition, and possible use of high-concentration substrate for the process feed, reducing process wastes and production costs. The use of extractive fermentation also allows the engineer to produce and recover a desired fermentation product in one continuous step. Nevertheless, extractive fermentation using conventional extractants suffers from severe solvent toxicity and a low separation factor, and may require formidably expensive solvent regeneration procedures. Extractive fermentation usually involves the use of a water immiscible solvent, which is usually toxic to cells.

Successful development of an extractive fermentation process requires careful selection of a highly efficient and non-toxic solvent or adsorbent for extraction. Most organic solvents are toxic to bacteria; they will either inhibit or stop bacterial growth. Solvent toxicity can exert on the microorganisms at the molecular level and at the phase level. Toxicity at the phase level comes from the direct contact of the solvent phase with the cells, which may block nutrient diffusion from the medium to cells due to solvent coating and may disrupt the cell wall due to increased surface tension. Toxicity at the molecular level comes from the dissolved organic solvent, which can inhibit enzymes or modify cell membrane permeability. Phase toxicity can be eliminated by using hydrophobic membranes or cell immobilization. Solvent toxicity at the molecular level can be reduced to a minimal degree by using solvents that are essentially insoluble in water. Immobilized cells were also found to be more resistant to solvent toxicity than free cells.

Several aliphatic amines have been used successfully to extract carboxylic acids, with long-chain tertiary amines receiving the most attention. The strong amine interactions with the acid allow for the formation of acid-amine complexes, and thus provide for high distribution coefficients. In addition, the high affinity of the organic base for the acid gives selectivity for the acid over non-acidic components in the mixture. The extractant can be regenerated by back-extraction with a strong acid or base, or by stripping. Several ion-exchange resin adsorbents also have been used to separate lactic acid from the fermentation broth. The adsorbed lactic acid can be easily stripped with hot water, base, or alcohol. The loading capacity of the adsorbent, however, is relatively low (<0.5 g/g resin) and requires frequent regeneration.

Inefficient phase separation is a major concern in large-scale extraction processes. Large amounts of solvent and product could be lost due to the formation of emulsion and poor phase separation, especially when the feed stream is "dirty" fermentation broth. This problem can be minimized by removing cells, cell debris, and proteinaceous materials from the broth using cell immobilization techniques during fermentation. The problem can also be reduced or eliminated by using a hollow-fiber membrane extractor (HFME), where the membrane separates feed and extractant and one of which wets the membrane (Basu and Sirkar, 1991). Solvent is usually contained in the shell side of the membrane extractor, while aqueous feed flows through hollow fibers. The large surface areas of the hollow fibers provide good phase contact and mass transfer. ME also overcomes the stability problems associated with the supported liquid membranes (SLM), where the extractant is contained in the membrane, which separates two aqueous phases. In extractive fermentation, solvent toxicity due to direct contact between cells and solvent can

also be eliminated by using the membrane extractor. Possible microbial contamination of the fermentor or cross contamination of the two streams was not found in ME.

We have studied the feasibility and advantages of using extractive fermentation for propionic acid production from lactose (Jin and Yang, 1998; Jin 1997). A mixture of Alamine 336 or Adogen 283 and 2-octanol or olyeal alcohol was used as the extractant. This extractant was somewhat toxic to the propionic acid bacterium. However, the immobilized cells in the fibrous-bed bioreactor showed much higher tolerance to solvent than the free cells present in conventional stirred-tank, batch fermentors. A three to five-fold increase in reactor productivity was attained in extractive fermentation compared with the one without extraction. The improved reactor productivity was attributed to proper pH control and reduced product inhibition through the removal of propionic acid by solvent extraction. A higher propionic acid yield, up to 50% increase from the conventional batch fermentation process, was also obtained from the extractive fermentation. Also, other fermentation byproducts, such as acetate and succinate, were significantly reduced in the extractive fermentation. These data have partially confirmed our hypothesis that in a heterogeneous (e.g., propionic acid) fermentation, a higher product (propionic acid) yield can be obtained due to metabolic shift caused by selective product (propionic acid) removal from the bioreactor.

Very little has been done on extractive fermentation for acetic acid production, especially with anaerobic homoacetic bacteria (San Nicolas et al., 1997). The requirement for a high pH around 7 for the growth of homoacetogens (Tang, 1986; Tang et al., 1989) presents a major challenge in developing an extractive fermentation (Yang, 1988).

III. ACETATE PRODUCTION FROM WHEY LACTOSE BY FERMENTATION

3.1 INTRODUCTION

Whey has been extensively studied as a fermentation substrate for producing lactic, propionic, and acetic acids. However, its use for anaerobic acetic acid fermentation has been limited because no homoacetogen can directly ferment lactose to acetate. Recently, the feasibility of producing acetate from whey fermentation using a co-culture consisting of homolactic and homoacetic bacteria has been studied in free-cell batch cultures (Tang et al., 1988) as well as in immobilized-cell continuous cultures (Yang et al., 1992). The homolactic and homoacetic bacteria sequentially convert lactose to lactate and then to acetate, with an overall acetic acid yield from lactose of ~0.9 g/g. However, in these studies, whey permeate was supplemented with large amounts of yeast extract and trypticase. Without these nutrient supplements, the fermentation was slow and conversion of whey lactose to acetate was also low. Yeast extract and trypticase are expensive to use in large-scale production of acetate from whey; thus, inexpensive nutrient supplements, such as corn steep liquor and industrial-grade yeast and casein hydrolysates, were studied in this work.

The economical use of a fermentation route for acetate production also depends on the reactor productivity and the final product concentration achievable from the fermentation (Busche et al., 1982). A high fermentation productivity usually can be achieved with an immobilized-cell bioreactor; however, conventional immobilized-cell bioreactors usually suffer from productivity loss over time when the cells are used continually or repeatedly in continuous or fedbatch fermentations. To overcome these problems, we have developed a fibrous-bed, immobilized-cell bioreactor for propionic acid and lactic acid fermentations (Silva and Yang, 1995; Yang et al., 1994; 1995). The fibrous-bed bioreactor gave stable, high-rate production of the fermentation product for long periods because of the high density of active cells maintained in the fibrous bed. Good fermentation results were also obtained with nonsterile whey permeate and acid whey without nutrient supplements when the fermentation was performed in the fibrous-bed bioreactor, but not in the conventional free-cell batch fermentation. A higher product concentration was also obtained with the fibrous-bed bioreactor operated in a recycle fedbatch mode (Yang et al., 1995). The fibrous-bed bioreactor has also been studied for continuous production of acetate from lactate and lactose in synthetic media (Yang et al., 1992). However, the highest acetate concentration ever attained in our previous studies was only ~40 g/L, which is considerably lower than the 80 g/L or higher attained in aerobic vinegar fermentation (Ebner and Follmann, 1983; Kondo and Kondo, 1996) and the anaerobic homoacetogenic fermentation of glucose by an acetate-tolerant strain of *C. thermoaceticum* (Parekh and Cheryan, 1994).

In Phase I, the feasibility of using the fibrous-bed bioreactor for long-term, high-rate production of acetate from lactose was evaluated at the laboratory scale. The two main objectives in Phase II study were: 1) to study the feasibility of producing a high concentration of acetate broth from whey fermentation with minimal nutrient supplements by using the fibrous-bed bioreactor, and 2) to scale up the bioreactor to a pilot scale and evaluate its performance.

3.2 CULTURES AND MEDIA

3.2.1 Microorganisms

Two homolactic acid bacteria, *Lactobacillus helveticus* (ATCC 15009) and *Lactococcus lactis* (formerly named *Streptococcus lactis*; OSU stock culture #588), were used in fermenting acid whey and sweet whey permeate, respectively. *Clostridium formicoaceticum* (ATCC 27076) was used for homoacetic fermentation.

3.2.2 Growth Media

Unless otherwise noted, the basal medium contained (per liter): 40 mL mineral #1 solution; 40 mL mineral #2 solution; 10 mL trace metals solution; 10 mL vitamin solution; 10 mL 0.005% $NiCl.6H_20$; 1 mL 0.2% $FeSO_4.7H_2O$; 0.5 mL 0.1% resazurin; 2 g trypticase; 2 g yeast extract; 6 g $NaHCO_3$. The mineral #1 solution contained 7.86 g/L $K_2HPO_4.3H_2O$. Mineral #2 solution consisted of (per liter): 6 g K_2HPO_4; 6 g $(NH_4)_2SO_4$; 12 g NaCl; 2.5 g $MgSO_4.7H_2O$; 0.16 g $CaCl_2.2H_2O$. The composition of trace metal solution was (per liter): 1.5 g nitrilotriacetic acid; 0.1 g $FeSO_4.7H_2O$; 0.5 g $MnSO_4.2H_2O$; 1.0 g NaCl; 0.1 g $CoCl_2$; 0.1 g $CaCl_2.2H_2O$; 0.1 g $ZnSO_4.5H_2O$; 0.01 g $CuSO_4.5H_2O$; 0.01 g $AlK(SO_4)_2$; 0.01 g H_3BO_3; 0.01 g $Na_2MoO_4.3H_2O$. The vitamin solution contained (per liter): 5 mg thiamine-HCl; 5 mg riboflavin; 5 mg nicotinic acid; 5 mg Capantothenate; 0.1 mg vitamin B_{12}; 5 mg p-aminobenzoic acid; 5 mg lipoic acid. Lactose was added to the basal medium for culturing homolactic bacteria, whereas lactate was added for homoacetic bacterium. Procedures used in preparing the synthetic medium can be found elsewhere (Tang, 1986; Zhu, 1992).

3.2.3 Fermentation Feedstocks

Acid whey (containing ~3.5% lactose and 0.8% lactic acid) was provided by Kraft General Foods from its Beaver Dam, Wisconsin, plant. Fresh acid whey was frozen and shipped to us overnight. They were sterilized by autoclaving at 121°C before use. Sweet whey permeate (WP) was obtained from Brewster Dairy, Inc. (Brewster, Ohio). Fresh sweet whey permeate received from Brewster Dairy was stored in a cold room at 4°C, usually for no more than 1 week. Whey permeate was sterilized by passing it through a 0.2 μm-pore size membrane filter before use.

Sterilized whey was used as the feed medium to homolactic fermentation and co-cultured fermentation. To promote the fermentation, WP and acid whey were supplemented with some nitrogenous sources, including yeast extract (Difco), trypticase (Difco), casein hydrolysate (UBC and Red Star), yeast hydrolysate (Red Star), and corn steep liquor (CSL). The corn steep liquor was received in a concentrated form (~45% total solids) from Cargill's corn wet-milling plant in Eddyville, Iowa. Before use, it was diluted with tap water by four-fold, heat sterilized at 121°C for 20 min, and the supernatant was then removed for use as a nutrient supplement to WP and acid whey in various volume fractions. CSL also contained small amounts of glucose, fructose, and lactic acid. Further information about these whey feedstocks can be found elsewhere (Silva and Yang, 1995; Huang, 1998).

3.3 FIBROUS-BED IMMOBILIZED BIOREACTOR

The following diagram shows the schematics of the fibrous-bed bioreactor and its spiral wound matrix structure. In this fibrous-bed bioreactor, cells were immobilized in a spiral-wound, fibrous matrix that provides large surface areas for cell attachment and large void spaces for cell entrapment to achieve high cell density ranging from 40 g/L to 100 g/L. Cell growth in the fibrous matrix can be controlled by limiting the growth nutrients present in the production medium. The bioreactor can be operated either continuously or as repeated batch or as fedbatch for a prolonged period. The large built-in vertical gaps among the spiral-wound layers of the fibrous matrix allow gases such as CO_2 and air to flow upward freely and escape from the top of the reactor, and the liquid medium and solid particles to be pumped through the reactor bed without substantial pressure drop.

3.3.1 Bioreactor Construction and Operation

The immobilized cell bioreactors were made of glass columns packed with spiral-wound terry cloth. Each reactor had a working volume of ~450 mL. Unless otherwise noted, these reactors were operated with high recirculation rate and pH control (see Figure 3.1). The reactor was operated with continuous feed or at recycle batch mode (see Figures 3.2 and 3.3). In the latter case, a 5-L stirred tank fermentor (Marubishi MD-300) with 3-L working volume was connected with the immobilized cell bioreactor. A positive pressure (3-5 psig) of N_2/CO_2 gas mixture was applied to the fermentor head space to maintain anaerobic condition in the system. Unless otherwise noted, the entire reactor system contained ~3.5 L of the medium and was maintained at 37°C and pH 7.5 by adding *6N* NaOH solution. Details on the startup and operation of these bioreactors can be found elsewhere (Yang et al., 1992).

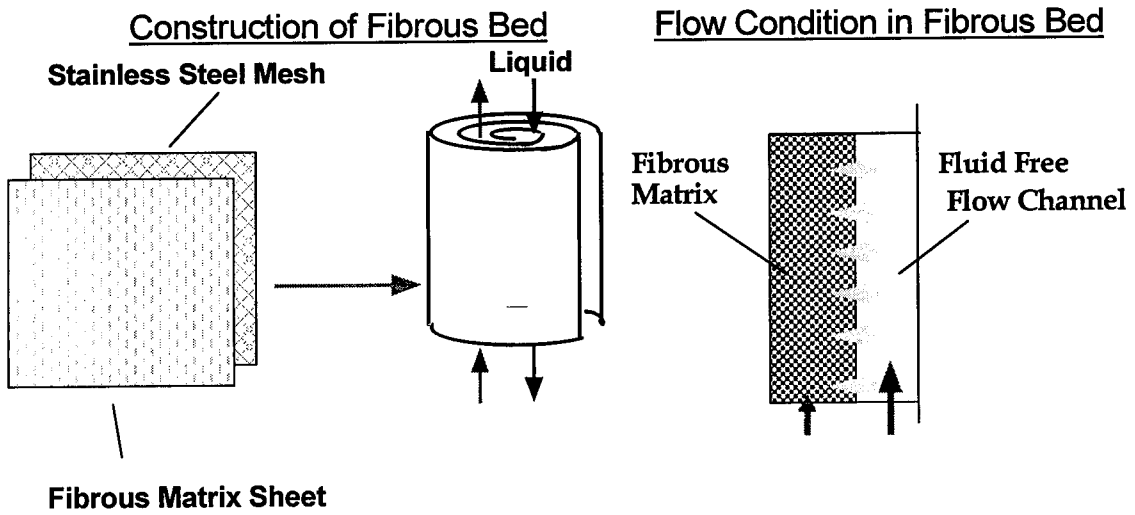


Figure 3.1. Construction of Convoluted Fibrous Bed and Flow Conditions in the Bed.

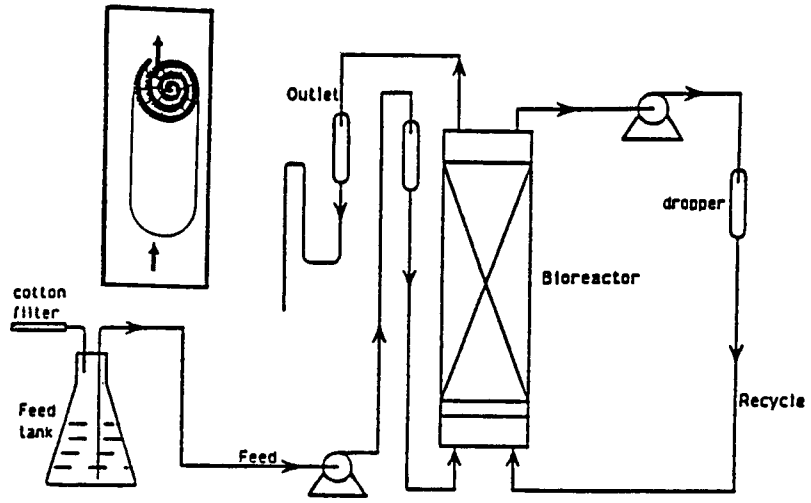


Figure 3.2. Continuous Immobilized Cell Bioreactor with Recirculation and pH Control.

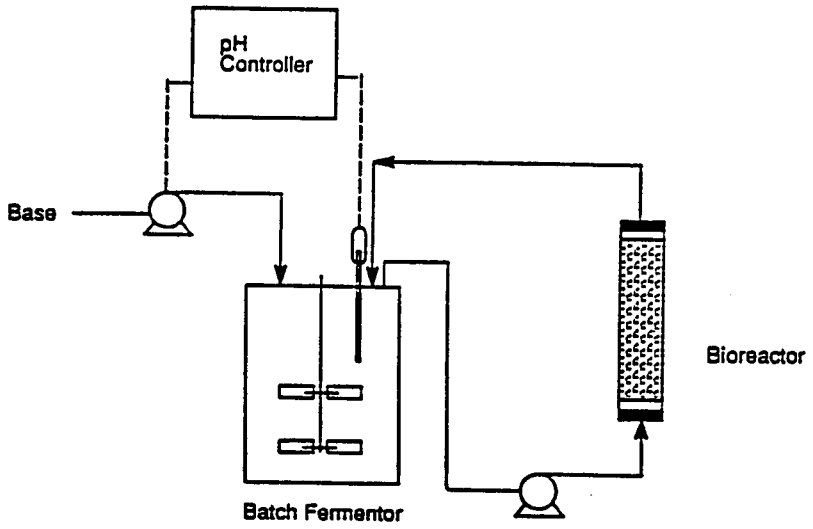


Figure 3.3. Recycle Batch Immobilized Cell Bioreactor System.

3.4 CONTINUOUS FERMENTATIONS

3.4.1 Homolactic Fermentations

Converting whey lactose to lactic acid via homolactic bacteria is relatively easy. *L. lactis* works well on whey permeate (Figure 3.4), while *L. helveticus* works well on acid whey (Figure 3.5). About an equal amount (>95% yield) of lactic acid was obtained from lactose in these fermentations, with complete conversion taking place at ~12 h retention time.

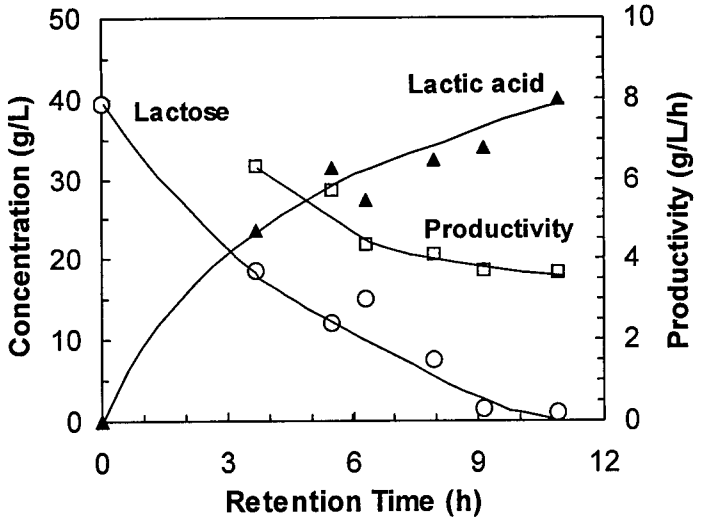


Figure 3.4. Continuous Homolactic Fermentation of Whey Permeate with Immobilized *L. lactis* Cells at pH 7, 37°C.

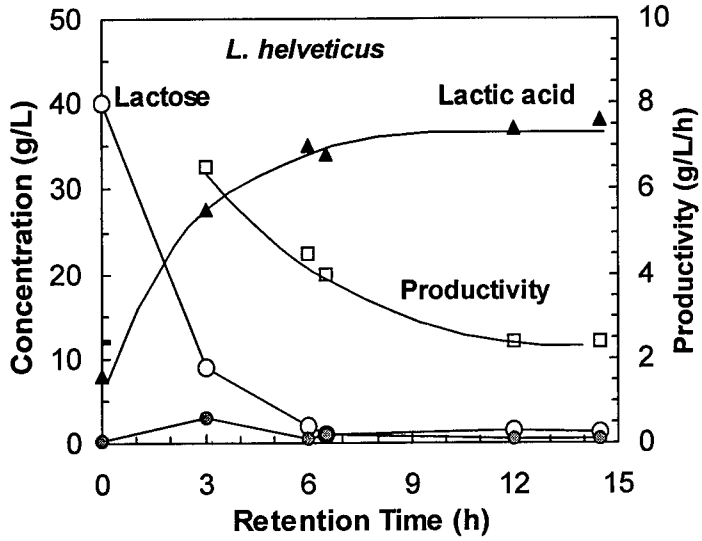


Figure 3.5. Continuous Homolactic Fermentation of Acid Whey with Immobilized *L. helveticus* Cells at pH 5.5, 42°C.

3.4.2 Homoacetic Fermentations

Continuous homoacetic fermentation of lactate in the synthetic medium was studied first. Typical results from immobilized cell bioreactors are shown in Figure 3.6. About an equal amount (>95% yield) of acetate was obtained from lactate in the reactor with pH control at 7.6. Complete conversion took place at ~45 h retention time. For a single-pass reactor without pH control, the reactor pH fell below 4 and fermentation ceased due to the acidic pH (Figure 3.7).

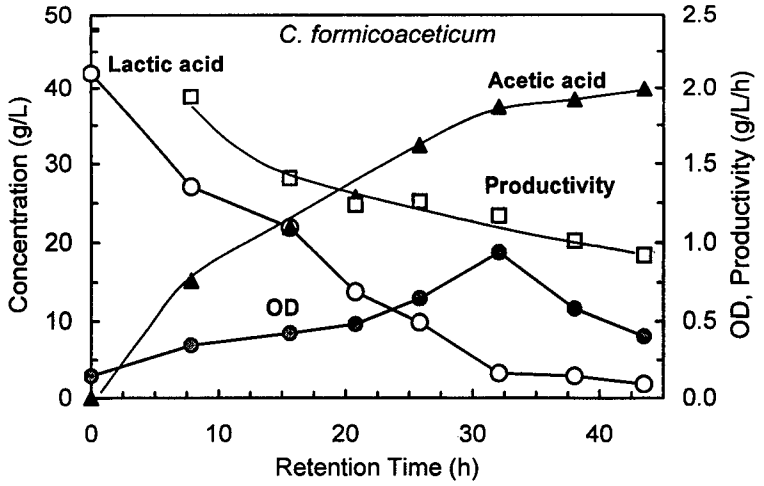


Figure 3.6. Continuous Homoacetic Fermentation of Lactate Synthetic Medium with Immobilized *C. formicoaceticum* Cells at pH 7.6, 37°C.(OD: optical density)

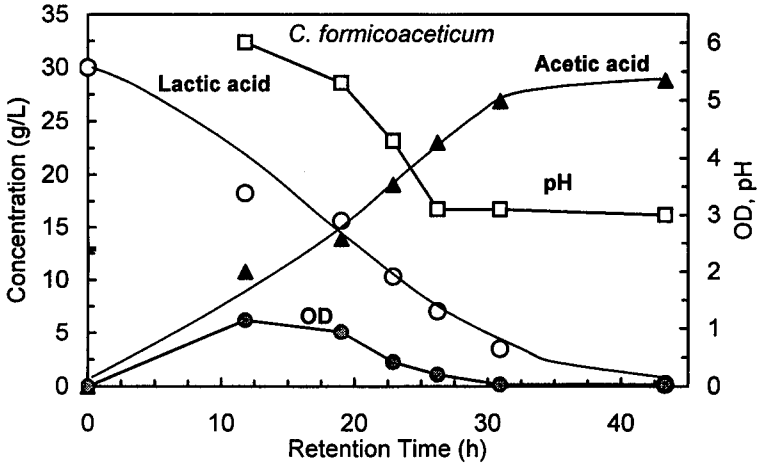


Figure 3.7. Homoacetic Fermentation of Lactate in a Single-Pass Bioreactor without pH Control. (OD: optical density)

3.5 FORMULATION OF WHEY MEDIA

Previous studies have shown that whey permeate was nutritionally sufficient for *L. lactis*, but not for *C. formicoaceticum* (Tang et al., 1988). For *C. formicoaceticum*, whey permeate without nutrient supplements gave poor cell growth. To develop a proper medium formulation for whey permeate as a feedstock for acetate production, the effect of various nutrient supplements on the growth of *C. formicoaceticum* was studied in serum tubes. Ten milliliters of whey media (pH 7.6) containing 10 g/L lactate and various amounts and types of nutrient supplements were placed in each serum tube. After inoculation, cell growth was followed by measuring the optical density (OD) of the culture tube in a spectrophotometer at 660-nm wavelength. The final medium pH and acetate content were also analyzed.

Preliminary screening experiments indicated that only yeast extract (Difco) supplementation was needed for good cell growth in whey permeate, whereas all other nutrients (e.g., trypticase, vitamins, trace metals, and mineral salts) used in the synthetic medium did not affect cell growth in whey permeate (data not shown). Yeast extract contains large amounts of organic nitrogen (amino acids and peptides), vitamins, minerals, and other growth factors, but is also relatively expensive. Therefore, several industrial-grade yeast hydrolysates (at 5 g/L), casein hydrolysate (at 5 g/L), and corn steep liquor (at 10% v/v) were also tested for their effect on cell growth. The results showed that all these supplements gave comparable cell growth rates to that from the control experiment using the more expensive yeast extract (Difco) in the synthetic medium.

Casein hydrolysate (CA) and corn steep liquor (CSL) were further evaluated to determine their appropriate supplement levels to whey permeate and their effects on the growth of *C. formicoaceticum*. As shown in Table 3.1, both CA (at 1.5 g/L) and CSL (at 0.8% v/v) were as good as yeast extract (at 2 g/L) in providing good cell growth and acetate production in whey permeate. In general, the fermentation was better at higher supplement levels. These results suggested that the amino acids present in yeast extract and trypticase are needed for whey media. These nutrients also can be provided from inexpensive sources such as casein hydrolysate and corn steep liquor.

Table 3.1. Growth Test of *C. formicoaceticum* in Whey Permeate with Various Nutrient Supplements.

Nutrient supplement	Growth[1]	Final pH	Acetate (g/L)
None	+/-	5.85	0.12
Yeast extract, 2 g/L	++	5.38	1.08
Corn steep liquor			
0.1% (v/v)	+	5.80	0.21
0.2%	+	5.61	0.37
0.4%	++	5.36	0.83
0.8%	++	5.49	1.08
1.6%	++	5.37	1.21
Casein hydrolysate			
0.25 g/L	+/-	5.32	0.10
0.5 g/L	+	5.27	0.10
1.0 g/L	++	5.20	0.54
1.5 g/L	++	5.38	1.50

[1]+/-: cell growth was not significant; +: significant cell growth; ++: good cell growth

The results were further confirmed with batch fermentations of acid whey, which was pre-fermented with homolactic bacteria to convert lactose to lactate. About the same fermentation rate and acetate yield were obtained from the synthetic medium and acid whey supplemented with 2 g/L yeast extract and 2 g/L trypticase, 2.5% (v/v) corn steep liquor, or 1.5 g/L casein

hydrolysate. On the other hand, acid whey without nutrient supplements yielded slow growth and low acetate production. Further work was conducted with co-cultured fermentation in sweet whey permeate discussed in the following section.

3.6 CO-CULTURED FERMENTATIONS

The reactor was operated with a high recirculation rate of ~300 mL/min in either batch or fedbatch mode. The reactor was first inoculated with *C. formicoaceticum* and allowed to grow for several weeks in the synthetic medium containing lactate as the carbon source. During this period, a high cell density of the homoacetogen was immobilized in the fibrous bed. The reactor was then inoculated with *L. lactis* and provided with yeast extract supplemented whey permeate as the substrate. This sequential inoculation procedure was used to ensure that the slower-growing *C. formicoaceticum* would not be outgrown by the faster-growing *L. lactis* in the co-cultured fermentation. After two batches, both bacteria were properly immobilized in the fibrous bed.

3.6.1 Batch Fermentations

Figure 3.8 shows two typical batch fermentations of whey permeate (WP) supplemented with 1.5 g/L casein hydrolysate (CH) and 2.5% (v/v) corn steep liquor (CSL), respectively. As shown in this figure, lactose was converted to lactate and then to acetate by the co-culture, with an overall acetic acid yield from lactose of ~0.9 g/g. The reactor productivity was ~0.24 g/(L·h) based on the total medium volume in the fermentation.

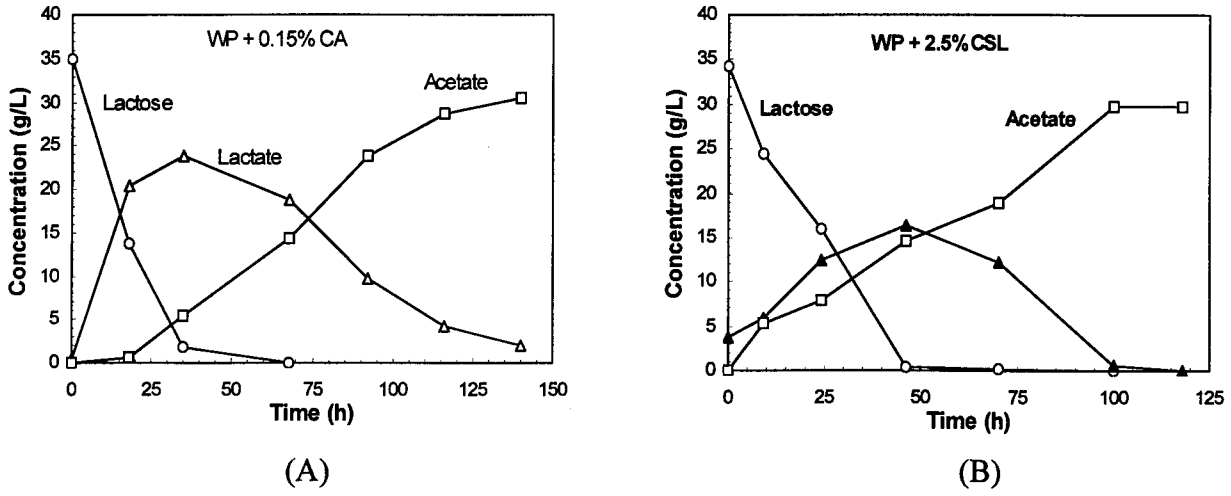


Figure 3.8. Kinetics of Acetate Production From Lactose by the Co-cultured Fermentation at pH 7.5, 37°C. (A) Whey Permeate Supplemented with 1.5 g/L Casein Hydrolysate, (B) Whey Permeate Supplemented with 2.5% (v/v) Corn Steep Liquor (40% T.S.).

As shown in Table 3.2, similar results were also obtained with other whey media, including supplemented acid whey. In all cases, supplementation with either 1.5 g/L CA or 2.5% CSL was sufficient to achieve fast fermentation and high conversion. The fermentation was poor when only 1% CSL was added to whey permeate. The optimal supplement level must be determined

with more experiments and based on the cost analysis. However, these experiments showed that efficient acetate production from whey lactose could be achieved with sweet whey permeate and acid whey supplemented with small amounts of inexpensive corn steep liquor or industrial-grade yeast and case hydrolysates using the fibrous-bed bioreactor operated in batch mode.

Table 3.2. Fermentations with Various Nutrient Supplements.

Medium	Initial Lactose/Lactate Conc. (g/L)	Final Acetate Concentration (g/L)	Acetate Yield[1] (g/g)	Productivity (g/(L·h))
Synthetic lactate medium				
2 g/L YE (Difco)	37.5	30.2	0.95	0.207
5 g/L YE (Amberex[2])	28.8	25.4	0.90	0.221
Whey permeate – with				
1.5 g/L CA	36.7	30.5	0.92	0.218
1% CSL	51.2	21.8	0.84	0.135
2.5% CSL	34.3	29.8	0.87	0.252
2.5% CSL	27.0	25.5	0.92	0.264
2.5% CSL + 1.5g/L CA	37.3	30.3	0.86	0.309
Acid whey – with				
2.5% CSL	21.0	20.4	0.90	0.231
Fedbatch fermentations				
WP w/ 2.5% CSL; 3.5 L	34.3	74.8	0.89	0.176
2 L	27.0	73.5	0.91	0.245

[1]acetate yield was based on the net production of acetate divided by net consumption of lactose or lactate.
[2]Amberex 1009 (Red Star, Milwaukee, WI) is a Brewer's yeast extract.
YE: yeast extract; CA: Casamino acid or hydrolyzed casein powder; CSL: corn steep liquor (with 40% total solids); WP: whey permeate.

3.6.2 Effects of Lactate

Lactate is the substrate for *C. formicoaceticum*. When the lactate concentration was lower than 20 g/L, the specific growth rate of the homoacetogen increased with increasing the lactate concentration in the medium. However, at higher lactate concentrations (>20 g/L), lactate inhibited the cell growth in the serum-tube study (data not shown).

Figure 3.9 shows the effect of lactate accumulation on acetate production in the co-cultured fermentation. In general, lactate production by the homolactic bacteria was much faster than acetate formation from lactate by the homoacetogen. Therefore, when the initial lactose concentration was higher, more lactate accumulation occurred during the co-cultured fermentation.

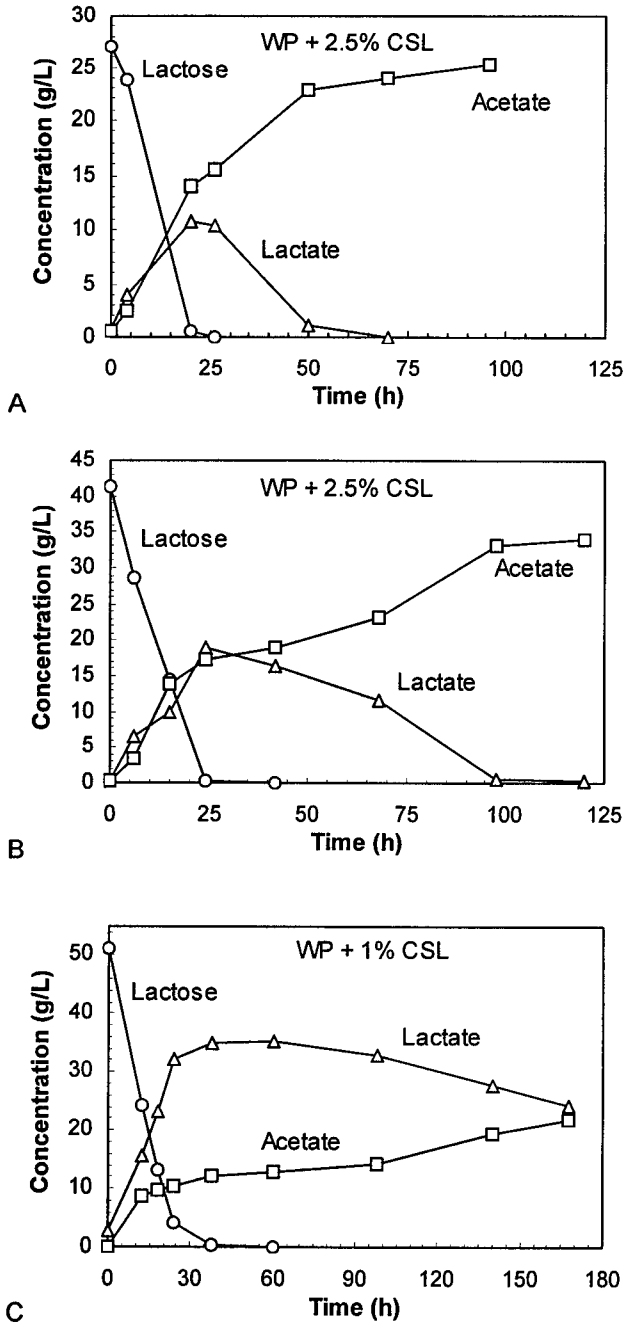


Figure 3.9. Effects of Lactate Formation and Nutrient Concentration on Acetate Production in the Co-cultured Fermentation at pH 7.5, 37°C. (A) WP (27 g/L Lactose) Supplemented with 2.5% (v/v) CSL, (B) WP (41 g/L Lactose) Supplemented with 2.5% (v/v) CSL, (C) WP (50 g/L Lactose) Supplemented with 1% (v/v) CSL.

The high concentration of accumulated lactate negatively affected acetate production in the co-cultured fermentation. As can be seen in Figure 3.9, the acetate production rate decreased significantly when large amounts of lactate accumulated in the medium, indicating a substrate inhibition to *C. formicoaceticum*. The inhibition effect of lactate on cell growth was also observed at concentrations higher than 20 g/L in the cell growth kinetic study. Therefore, it is important to keep the lactate concentration lower than 20 g/L to allow for a good acetate production rate. This would limit the initial lactose concentration in the whey media unless balanced lactate formation and consumption rates could be otherwise maintained throughout the co-cultured fermentation.

Lactate accumulation and inhibition were more severe when there was less nutrient supplementation (Fig. 3.9c). This was because the homoacetic fermentation depended more on additional nutrients than the homolactic fermentation. At low supplement levels, the homoacetic fermentation would be much slower than the homolactic fermentation, resulting in more lactate accumulation in the cocultured fermentation. Substrate (lactate) inhibition was also found with continuous cultures (Yang et al., 1992) and other homoacetic fermentation studies with *C. thermoaceticum* (Brumm, 1988). Because it adds the lactose in small doses over time, lactate does not accumulated to inhibitory levels in a fedbatch fermentation. Therefore, to attain higher acetate concentrations, fedbatch fermentation was recommended and studied.

3.6.3 Fedbatch Fermentations

Figure 3.10 shows two fedbatch fermentations of whey permeate supplemented with 2.5% corn steep liquor. As shown in this figure, steady acetate production to reach a high acetate concentration was achieved in the fedbatch fermentation. Lactate accumulation and inhibition were reduced to a minimal level, if any, by periodic addition of lactose. The highest acetate concentration reached in the fermentation was ~75 g/L when the fermentation stopped, probably because of product (acetate) inhibition. However, this acetic acid concentration is the highest level ever produced by *C. formicoaceticum* in either pure culture using lactate as the substrate or co-culture with *L. lactis* using whey or lactose as the substrate.

The volumetric productivities and acetate yields were determined for batches corresponding to each of the five lactose addition periods during the fedbatch fermentation. As also shown in Figure 3.10, acetate productivity gradually decreased with time as the acetate concentration increased to 70 g/L. The productivity decreased dramatically at the acetate concentration higher than 70 g/L, indicating a strong product inhibition at this level. The overall productivity based on the total liquid volume was 0.176 g/(L·h) for the 3.5-L run and 0.245 g/(L·h) for the 2-L run. Acetate yield remained almost constant throughout the fedbatch fermentation. The overall acetic acid yield from lactose was ~0.9 g/g, similar to the ones from batch fermentations.

The second fedbatch fermentation (Figure 3.10B) was faster than the first one (Figure 3.10A) because the total medium volume was smaller in the second one. Based on the fibrous-bed bioreactor volume, the overall acetate productivity in the fedbatch fermentation was 1.23 g/(L·h) for the first one and 1.13 g/(L·h) for the second one. The similar reactor productivities indicate that most reactions occurred in the fibrous-bed bioreactor, instead of in the fermentor. This is consistent with the fact that most cells were immobilized in the fibrous bed. It was found at the

end of this study that less than 20% of the total cell population was present as suspended cells in the fermentation broth.

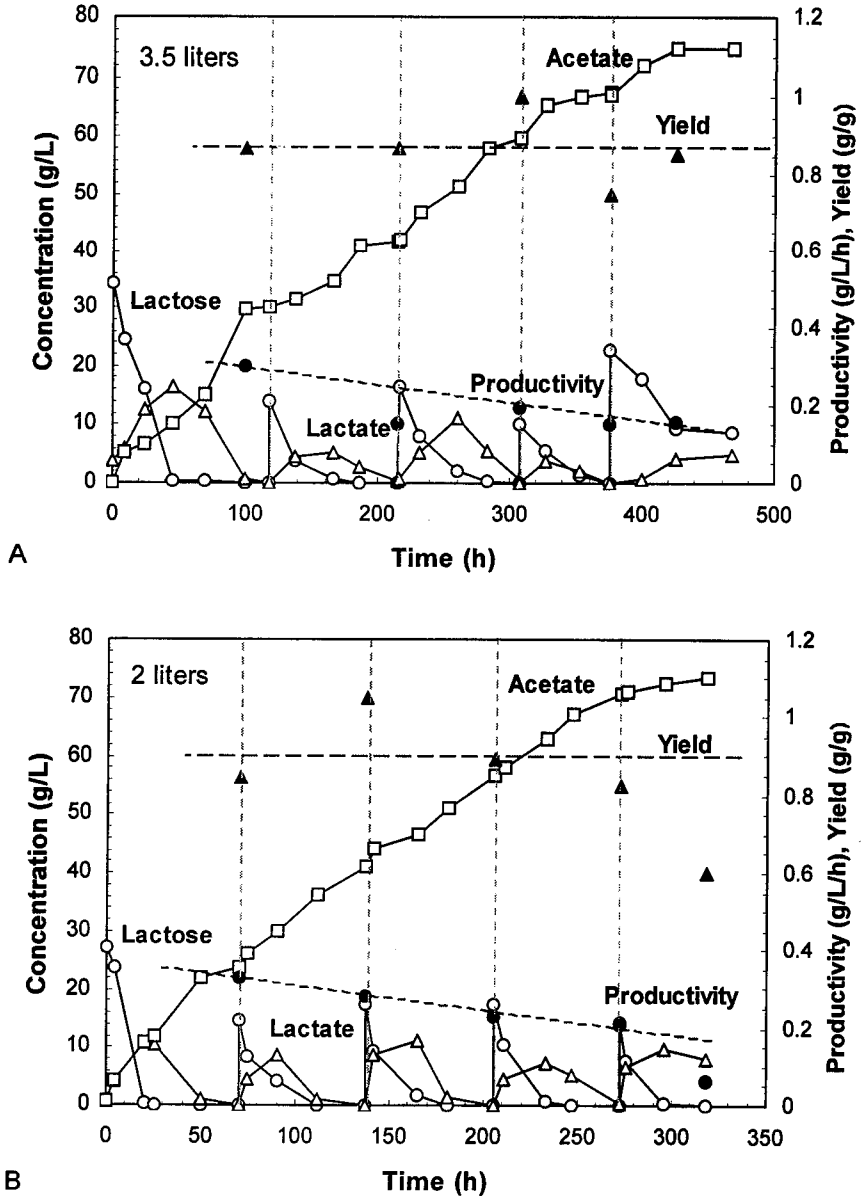


Figure 3.10. Kinetics of Fedbatch Fermentations of Whey Permeate Supplemented with 2.5% (v/v) Corn Steep Liquor at pH 7.5, 37°C. (A) 3.5-L Total Medium Volume, (B) 2-L Total Medium Volume. (▲ - yield, ● - productivity, □ - acetate, △ - lactate, ○ - lactose)

It should be mentioned that high acetate concentrations also inhibited the homolactic bacteria, as can be seen from the decreased lactose consumption rate at increased acetate concentrations (Fig.3.10). However, this inhibition should not have any negative effect on the overall

fermentation rate because the homolactic acid fermentation was still faster than the homoacetic acid fermentation. In fact, this inhibition might have helped to balance the formation and consumption of lactate in the co-cultured fermentation by slowing down the homolactic fermentation to a rate closer to the homoacetic fermentation.

As shown in this study, either CA or CSL can be used to supplement whey permeate or acid whey for their efficient use as economical feedstocks for acetate production by the cocultured fermentation. To our best knowledge, this is the first successful demonstration of anaerobic acetate production with a high yield from an industrial-grade feedstock. All previously reported anaerobic homoacetic fermentations used synthetic media containing a high concentration of yeast extract (Shah and Cheryan, 1995), which is cost-prohibitive for industrial fermentation. The low-cost nutrient supplements and feedstocks are important to the economical production of a low-cost acetate deicer.

However, there are cautions in using the relatively crude industrial feedstocks. In this work, we encountered some contamination problems with corn steep liquor as a supplement to whey permeate. Frozen-stored CSL was difficult to sterilize because it contained a high number of bacterial spores. Incomplete sterilization of the medium resulted in poor fermentations due to contamination by a butyrate producer, which outgrew the homoacetogen in the contaminated batch fermentation and resulted in a low acetate yield of only ~0.65 g/g (data not shown). The contaminant was probably a spore-forming *Clostridium* because its spores were hard to inactivate in autoclaving. The butyrate produced by this organism also inhibited the homoacetic bacterium. Under this situation, CSL must be autoclaved twice, with a one-day period between the two autoclavings to allow the spores to germinate. However, this stringent sterilization procedure was not required when fresh CSL was used in preparing the whey media.

There are many advantages to using the co-culture and the fibrous-bed bioreactor in the fermentation. Under fedbatch fermentation conditions, a high acetate concentration was produced from the immobilized-cell fermentation. The highest acetate concentration previously obtained from free cells of *C. formicoaceticum* grown on lactate was only ~40 g/L (Yang et al., 1992). In this work, 75 g/L of acetate concentration and a reactor productivity of 1.23 g/(L·h) were obtained. Even higher productivity is possible with higher nutrient supplements and a higher cell density in the fibrous-bed bioreactor.

Parekh and Cheryan (1994) reported that 83-100 g/L of acetate was produced from glucose by a mutant strain of *C. thermoaceticum* in a fedbatch fermentation. The acetate yield was only 0.74 ~ 0.8 g/g glucose and the productivity was 0.6-0.85 g/(L h). Improved productivity was also reported by the same research group with a two-stage continuous fermentation process with cell recycle and at a lower acetate concentration of ~35 g/L (Shah and Cheryan, 1995). However, a complex synthetic medium with excess nutrients and cell recycle with a hollow-fiber membrane filter were used to maintain a high viable-cell density in their study. Similarly, high acetic acid concentration (up to 90 g/L) and productivities were also produced from aerobic fermentation of ethanol by *Acetobacter aceti* in a repeated fedbatch culture with cell recycle (Park et al., 1991a). However, cell viability was very low at acetic acid concentrations higher than 60 g/L. A two-stage fermentation process was thus suggested to partially overcome this problem (Ito et al., 1991). However, vinegar fermentation has a low acetic acid yield from glucose, ~0.6 g/g or

lower, and requires extensive aeration. Furthermore, using a hollow-fiber membrane filter for cell recycle to achieve a high cell density and reactor productivity in these studies could be a major problem for long-term operation and process scale-up because dead cells accumulate and filtrate flux declines with time. The fibrous-bed bioreactor gave a comparable fermentation performance and would not be subjected to membrane fouling and other problems associated with the hollow fiber.

The good fermentation performance of the fibrous-bed bioreactor can be attributed to its abilities to maintain a high cell density (~30 g/L) in the bioreactor and to adapt the cells to tolerate a high acetate concentration. With the high cell density in the fibrous bed, fermentation no longer depends on fast cell growth in the batch reactor to achieve a high productivity. Consequently, a lower nutrient supplement level can be used compared with that required for the free-cell fermentation system. A previous study by Tang et al. (1988) used 8 g/L yeast extract to supplement whey permeate but could only get ~20 g/L acetate with 50% substrate conversion. Also, the high cell density allowed the reactor to better resist contamination. As mentioned before, improper sterilization of CSL could seriously reduce acetate production. However, the reactor with a high cell density in the fibrous bed could be easily recovered from the contamination by changing the contaminated medium to a new, properly sterilized medium in the fermentor. The fermentation rate and acetate yield returned to normal levels after replacing the medium (data not shown). This indicated that the fibrous-bed bioreactor could tolerate a low contaminant level. Previous studies with this type of bioreactor have also indicated that non-sterile media could be used as the feed to the reactor as long as the contaminant level in the medium was not high and would not take over the bioreactor (Silva and Yang, 1995; Yang et al., 1995).

3.7 ACETATE TOLERANCE

Another advantage of the fibrous-bed bioreactor is its ability to quickly adapt cultures to tolerate a high acetate concentration. At the end of this study, cells removed from the fibrous-bed bioreactor were tested for their growth tolerance to acetate under suspended-cell culture condition and the results were compared with those from the original culture used to seed the bioreactor. As shown in Figure 3.11, cells from the fibrous-bed bioreactor had a much higher tolerance to acetate than the original culture, as indicated by their higher specific growth rate and lower sensitivity to acetate inhibition. A previous study also showed that the highest acetate concentration allowing for cell growth was ~50 g/L (Tang et al., 1989). These results suggest that the fibrous-bed bioreactor provided an environment suitable for adapting and enriching acetate-tolerant strains. Similar results were also found with propionic acid fermentation performed in the fibrous bed bioreactor (Yang et al., 1995).

It is desirable to use acetate-tolerant mutants to produce a high acetate concentration in the fermentation. Mutants used in fermentation were usually induced with chemical mutagenic agents or ultraviolet light, and then selected with selective enrichment procedures (Parekh and Cheryan, 1991). However, this procedure is tedious and it could take a long time to find a useful mutant. Reed et al. (1987) developed a continuous selection process in a bioreactor environment incorporated with UV irradiation and a chemical mutagen, which killed most cells in the bioreactor. In this work, acetate-tolerant strains were obtained in the bioreactor without using

mutagens. Only a selection pressure (that is a high acetate concentration) was needed to obtain the mutant in a relatively short period. It is speculated that the environment provided in the fibrous bed might have been an important factor in getting the acetate-tolerant mutant. Figure 3.12 shows the effects of acetate concentration on the reactor productivity and acetate yield from lactate, and on the specific growth rate obtained from the growth kinetics study. It is clear that cells had good tolerance to acetate until the acetate concentration was higher than 7%. These results are consistent with those from the co-cultured fermentation with lactose as the substrate. The acetic acid yield from lactate was ~0.95 g/g lactic acid and also was not affected by the acetate concentration.

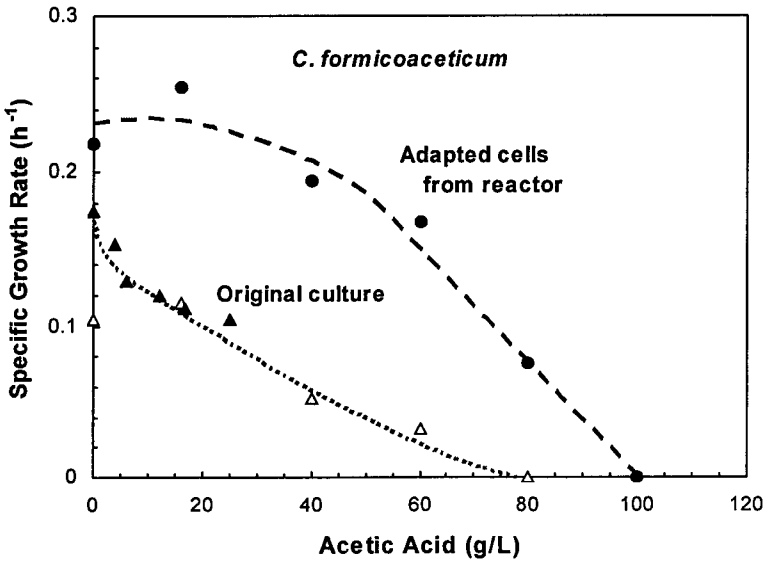


Figure 3.11. Effects of Acetic Acid Concentration on the Specific Growth Rates of the Adapted Cells from the Bioreactor and the Original Culture of *C. formicoaceticum.* (▲ - data from Tang et al., 1989; △ , ● - data from this study)

3.8 ANAEROBIOSIS

Homoacetogens are strict anaerobes; they cannot grow well in the presence of trace oxygen. In pure culture, *C. formicoaceticum* and other homoacetogens, such as *C. thermoaceticum*, must be cultivated in a medium in which the oxygen content has been purged with nitrogen gas and the redox potential reduced to a negative value with a reducing agent, such as cysteine. This anaerobiosis growth requirement could be a serious problem in large-scale homoacetic fermentations. This problem, however, was overcome by using the co-cultured fermentation. Co-immobilization with the homolactic bacterium in the fibrous bed removed the requirement for gas purging or chemically reducing the whey medium. The homolactic acid bacterium, which is not as sensitive to oxygen as the homoacetogen, might have removed oxygen, thus reducing the redox potential to a level suitable for the strict anaerobe, homoacetogen.

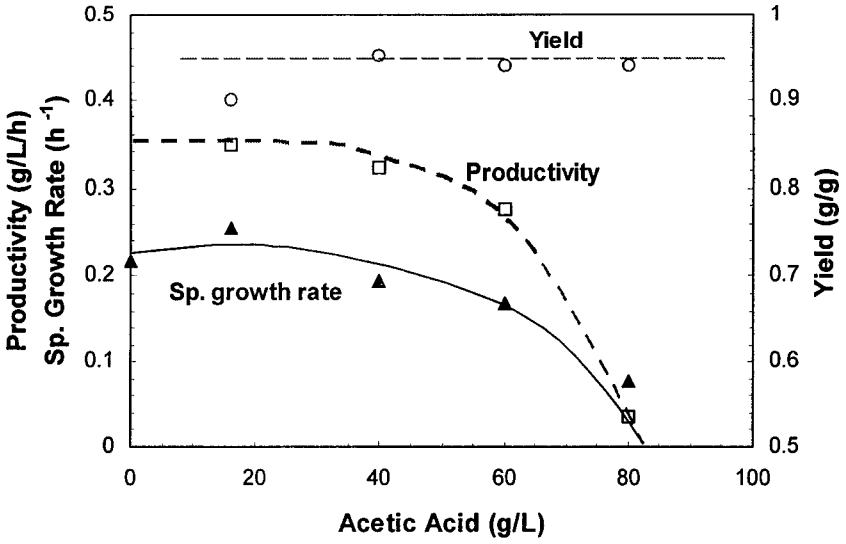


Figure 3.12. Effects of Acetic Acid Concentration on Acetic Acid Yield, Productivity, and the Specific Growth Rate of the Homoacetogen Grown in the Fibrous-Bed Bioreactor.

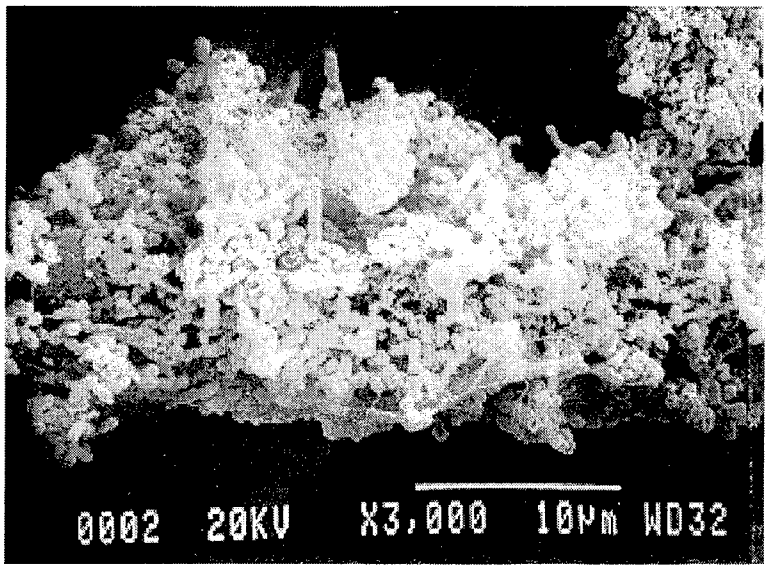


Figure 3.13. Scanning Electron Micrographs of *C. formicoaceticum* (Long Rods) and *L. lactis* (cocci) Co-immobilized on the Fiber Surface in the Fibrous-Bed Bioreactor.

A scanning electron micrograph (Figure 3.13) shows that the homolactic bacteria (cocci) and the homoacetic bacteria (long rods) co-lived in the fibrous bed. Most of them colonized together on the surface of the fiber. Although it cannot be easily seen in Figure 3.13, other SEM photos showed that most of *C. formicoaceticum* cells were attached directly to the fiber surface and were

then covered by *L. lactis* cells, which did not attach to the fiber surface as well. This kind of colonization is consistent with the immobilization sequence used in the reactor startup and explains why the homoacetogens can survive in the medium without reducing its redox potential. Apparently, these two bacteria have developed an intimate relationship for both to survive well in the bioreactor.

3.9 REACTOR LONG-TERM STABILITY

The immobilized cell bioreactors were found to be very stable in their performance, operated at either sequential recycle batch or continuous mode. These reactors were also able to accept non-sterile whey feed without significant degeneration or contamination problems. Some of the reactors have been operated continuously for more than 6 months. This excellent stability and high productivity of the immobilized cell bioreactors can be attributed to the high cell densities (up to 60 g/L) achieved in reactors and the immobilization mechanism provided by the spiral-wound fibrous bed.

3.10 EFFECT OF CATIONS

Three fedbatch homoacetic fermentations with three different bases, NaOH, $Ca(OH)_2$, and $Mg(OH)_2$, to control the pH were studied. As shown in Figure 3.14, all three batch fermentations had comparable performance, indicating that these cations have similar effects on fermentation. It is also noted here that the highest acetate concentration attained in the fedbatch free-cell fermentations was less than 40 g/L, which was significantly lower than that obtained with the immobilized cell bioreactor.

Recycle batch fermentations also have been conducted using CaO/MgO slurry to neutralize acetic acid to produce CMA directly from whey. After removing cells and unreacted CaO/MgO particles, the whole broth was oven-dried at 104°C to obtain unrefined CMA deicer.

3.11 FERMENTATION SCALE-UP

In Phase II, significant effort and time were spent to design, construct, and test the performance of a pilot-scale fermentation system. The objectives of the pilot plant study were to ensure that a similar fermentation result can be attained with the pilot-scale bioreactor and to discover and solve any potential operation difficulties in running a large-scale fermentation process for acetate production from whey lactose.

3.11.1 Equipment

A computer-controlled 150-L fibrous-bed bioreactor was designed and built for this project study. Detailed design and description of the bioreactor system is given in Appendix A. The engineering design factors important to the scale-up of the fibrous bed bioreactor can be categorized in three areas: 1) those affecting hydrodynamic and mixing performance, 2) those affecting cell immobilization (adsorption to and desorption from the fibrous bed), and 3) those affecting the fermentation kinetics. Fermentation kinetics ultimately will be used to evaluate the bioreactor and fermentation performance, and thus will be the focus of this report.

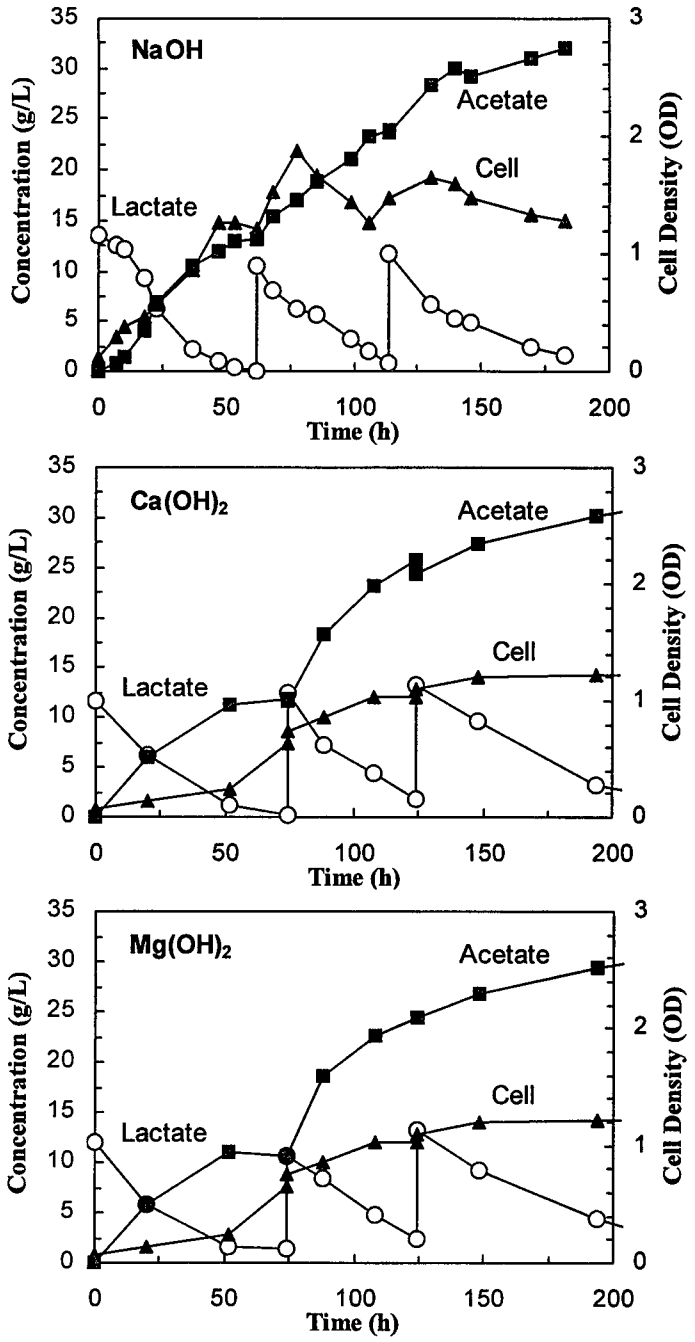


Figure 3.14. Fedbatch Homoacetic Fermentations with NaOH, $Ca(OH)_2$, and $Mg(OH)_2$ to Control the pH.

In addition to the 150-L bioreactor system, we also had to design and build a membrane sterilization system to process the whey feed. Details about the membrane system developed for this purpose are given in Appendix B. The membrane sterilization procedure is not necessary for a future large-scale plant located near the whey source, however.

3.11.2 Reactor Start-Up

Reactor start-up was a critical step toward the successful operation of the pilot-scale fermentation. As mentioned in the laboratory study section, the reactor should be seeded first with the homoacetogen, *C. formicoaceticum*, which has a lower fermentation rate than the homolactic acid bacteria and would determine the overall performance of the co-cultured fermentation. In the laboratory reactor study, lactate medium was used to start up the bioreactor; however, lactate was expensive to use at pilot and production scales. Therefore, an alternative carbon source, fructose, was studied and used for growing *C. formicoaceticum* during reactor start-up. Once the reactor had been successfully started up, *L. lactis* was inoculated to the bioreactor and whey permeate was used as the fermentation substrate.

3.12 HOMOACETIC FERMENTATION OF FRUCTOSE

Although we had extensively studied *C. formicoaceticum* for many years, no fermentation kinetics has been studied with fructose as the carbon source. In addition to the start-up issue, fructose is also of interest because corn steep liquor also contains a significant amount of fructose. Figure 3.15 shows typical fermentation kinetics with fructose as the substrate in a free-cell fermentation system. Acetic acid was the major fermentation product from fructose, with close to 100% yield. A strong acetic acid inhibition on cell growth was observed when acetate concentration reached ~20 g/L, as evidenced by the leveling of cell density (OD) in the fermentation broth.

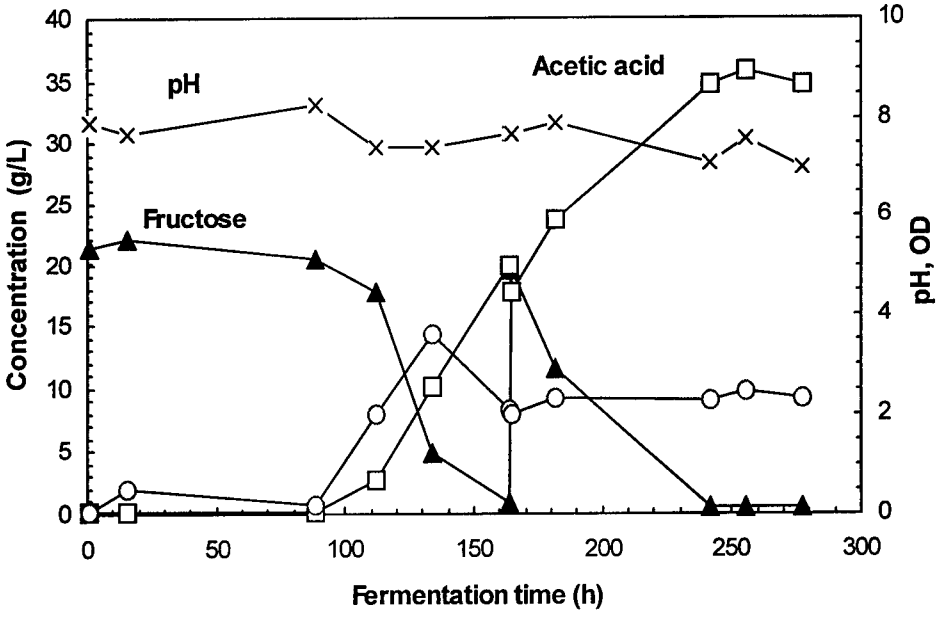


Figure 3.15. Kinetics of Homoacetic Fermentation with Fructose as the Substrate in a Free-Cell System. (OD: optical density)

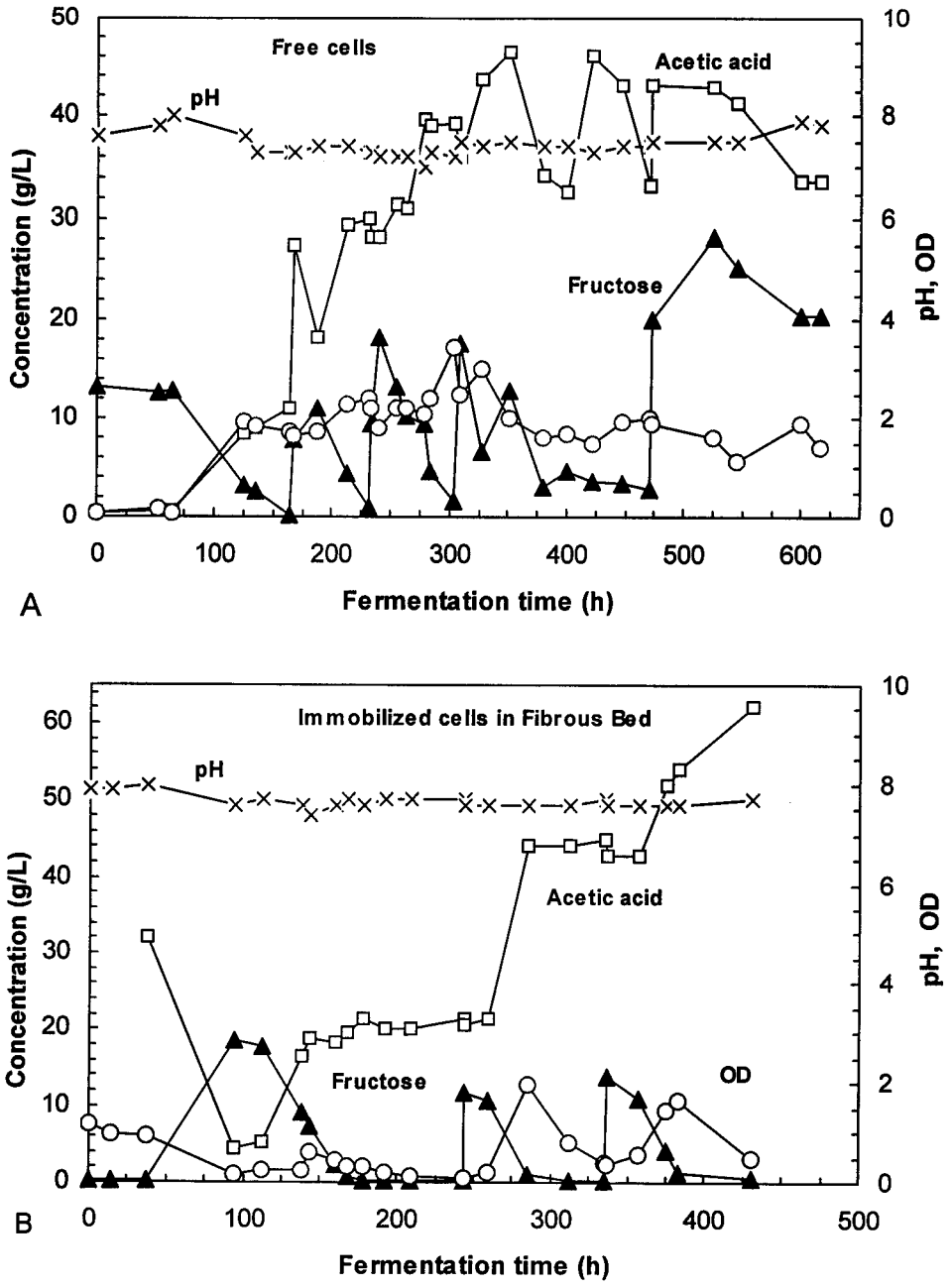


Figure 3.16. Kinetics of Fedbatch Homoacetic Fermentation with Fructose as the Substrate: (A) Free-Cell System; (B) Immobilized Cells in the Fibrous-Bed Bioreactor.

Figure 3.16 shows the fedbatch fermentations with fructose as the substrate in both a free-cells system and in the fibrous-bed bioreactor system. As can be seen in this study, the fibrous bed bioreactor gave a higher productivity and higher acetate concentration, 60 g/L or possibly higher, than those from the free-cells fermentation (acetate concentration less than 45 g/L). This result is consistent with prior study with lactate or lactose as the substrate for acetate production (Huang and Yang, 1998).

3.13 REACTOR START-UP PERFORMANCE

Figure 3.17 shows the start-up of the 150-L bioreactor with fructose medium. The reactor was operated as a batch reactor for the first 150 h to allow cells to grow and become immobilized to the fibrous bed. The medium in the bioreactor was then replaced with fresh medium for a second batch. After the second batch, whey medium was put into the bioreactor along with the inoculation of *L. lactis*. The fermentation kinetics with whey lactose is shown in Figure 3.18. As expected, the acetate yield from fructose and lactose was close to 100%.

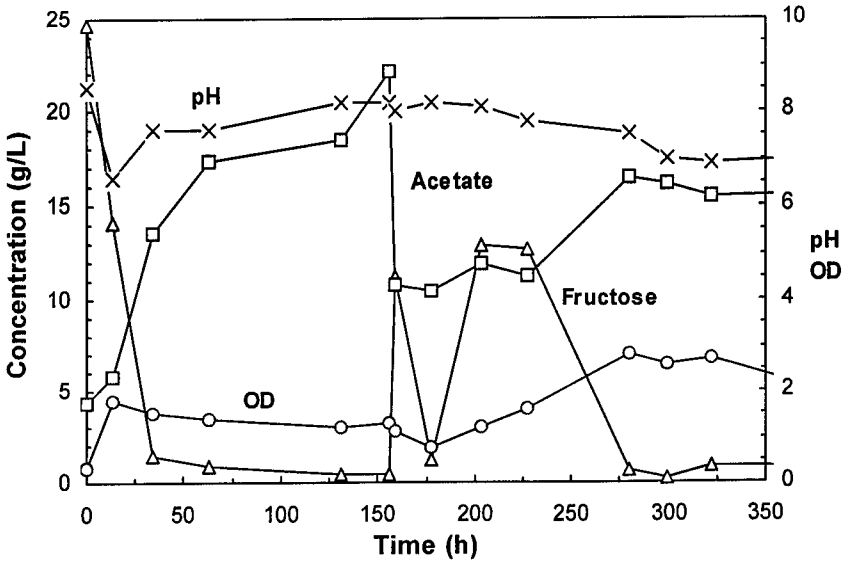


Figure 3.17 Start-Up of 150-L Bioreactor with Fructose Medium to Grow *C. formicoaceticum* in the Fibrous Bed.

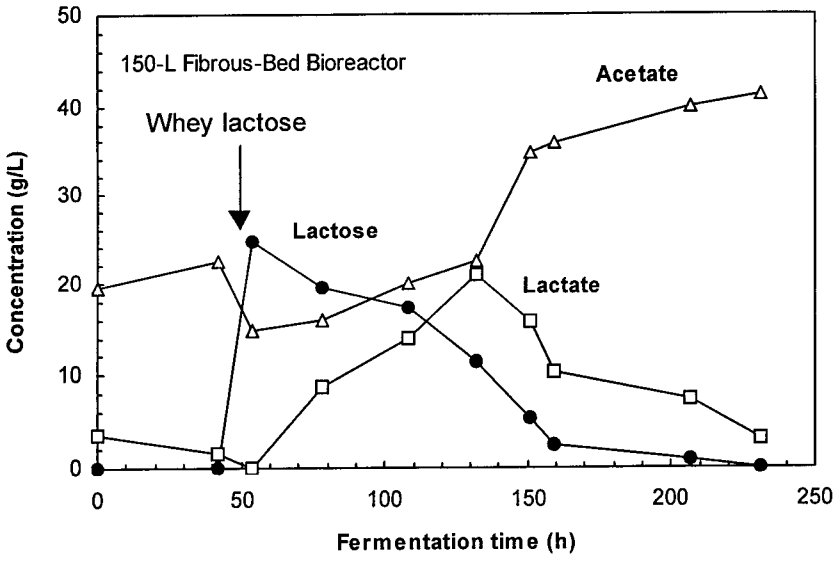


Figure 3.18. Kinetics of Co-cultured Fermentation to Produce Acetate from Whey Lactose in the 150-L Fibrous-Bed Bioreactor.

In general, the fermentation kinetics in the 150-L bioreactor were similar to those obtained in the laboratory bioreactor (4 L volume), though the productivity in the 150-L bioreactor was lower, ~0.15 g/L·h as compared with 0.4 g/L·h for the laboratory reactor. The lower productivity from the 150-L bioreactor was attributed to the lower cell density in the bioreactor, which was still in the early stage of its start-up and had not reached its maximum cell density.

3.14 CONCLUSION AND SUMMARY

Whey permeate and acid whey can be used as feedstocks to produce acetate using the co-culture immobilized in the fibrous-bed bioreactor with corn steep liquor, casein hydrolysate, or industrial-grade yeast extracts as nutrient supplements. About 0.9 g of acetic acid was produced from each gram of lactose consumed in the co-cultured fermentation. A high acetate concentration of 75 g/L was attained in fedbatch fermentation. Fedbatch operation adapted the culture to tolerate high acetate concentrations and induced and enriched acetate-tolerant strains in the bioreactor. The fermentation rate decreased only slightly with increasing acetate concentration up to ~70 g/L, then dropped dramatically beyond 70 g/L. At 75 g/L acetate, the overall productivity was ~0.23 g/(L·h) based on the total medium volume and 1.13 g/(L·h) based on the fibrous-bed reactor volume. The maximum acetate concentration that the bacteria could tolerate was ~80 g/L. The homoacetic bacteria were also inhibited by a high lactate concentration (>20 g/L) accumulated in the co-cultured fermentation. The lactate inhibition problem was also overcome by using fedbatch fermentation to maintain balanced lactate formation and consumption.

In this work, lactose was used as additional substrate in the fedbatch fermentation study. For industrial production purposes, concentrated acid whey or de-lactose whey permeate, which have high total solids and lactose concentration (up to ~200 g/L), can be used in the fedbatch fermentation process. In conclusion, acetate can be produced from whey lactose with a minimal nutrient supplementation with corn steep liquor using the fibrous-bed bioreactor. The acetate yield from lactose is 90% or higher, the final acetate concentration from the fermentation is ~4% or higher, and the immobilized cell bioreactor has a productivity of 1~2 g/L·h, depending on the acetate concentration. The reactor is very stable for long-term operation, either as a continuous reactor or operated as a recycle batch reactor. Further improvement in reactor performance can be attained by using extractive fermentation to remove the inhibiting acetic acid product from the fermentation medium, which will be discussed later in this report.

The pilot-scale bioreactor showed a similar kinetic performance to the laboratory bioreactor, indicating that the fermentation technology using fibrous-bed bioreactor would be applicable for production purposes, though more detailed testing and optimization studies are needed.

These fermentation results indicate that commercial production of acetate from low-cost industrial feedstocks (e.g., whey permeate) and nutrient supplements (e.g., CSL) is feasible. Thus, the fermentation process should have a good potential to economically manufacture CMA at a cost significantly lower than the current cost of CMA.

IV. RECOVERY AND SEPARATION OF ACETIC ACID FROM FERMENTATION BROTH BY EXTRACTION

The objective of this study was to develop a cost-effective method to recover and separate acetic acid from the fermentation broth. During Phase I, a two-step extraction process involving extraction with an amine-based extractant and back-extraction with a base solution was investigated using a shake test and a packed-column continuous extractor. The results showed that the process concept is feasible and energy-efficient; however, some difficulties were also identified. The major challenges include: 1) anions present in the fermentation broth would interfere with extraction, and 2) the relatively high pH value in the fermentation broth made extraction inefficient. In Phase II, extraction of acetic acid in a pilot-scale Karr column was studied. In addition, research focused on: 1) understanding the amine extraction chemistry to find methods to overcome the anion interference and pH problems, and 2) develop a hollow-fiber membrane extractor (HFME) for use in extractive fermentation. Emphases were placed on enhancing extraction rate and selectivity for acetic acid in the acetic/lactic acids mixture system. The experimental methods and results are discussed in this section.

4.1 BATCH EXTRACTION (SHAKE TEST)

4.1.1 Solvents

Four aliphatic amines, Alamine 336 (Henkle Corp.), Aliquat 336 (Henkle Corp.), Adogen 283 (Witco), and Amberlite L-1 (Rohm & Haas), were studied for their abilities to extract (separate) acetic acid from aqueous solutions, including fermentation broth. Three diluents, 2-octanol, oleyl alcohol, and diisobutyl ketone, were studied for their abilities to improve extraction performance.

4.1.2 Batch Extraction

Batch extraction was conducted in test tubes to determine the extraction coefficients, K_D, for various solvents under different conditions. Unless otherwise noted, equal volume of the aqueous solution containing acetic acid and the solvent extractant were mixed vigorously at room temperature for several minutes, and then centrifuged to separate the two phases. Back-extraction of the extractant was done with NaOH solution. The lactate concentration in the aqueous phase was analyzed with high performance liquid chromatography (HPLC). Similar extraction experiments were also conducted with lactic acid for comparison purposes.

The K_D values under various conditions for four amine extractants studied are shown in Table 4.1. Adogen 283 has the highest K_D values for both lactic acid and acetic acid. However, Alamine 336 has a better selectivity for lactic acid over acetic acid than the other amines. About the same K_D value was obtained with whey or water, indicating the impurities present in whey did not interfere with the extraction. However, as also shown in Table 4.1, the distribution coefficient was lower when the aqueous phase also contained other anions, such as chloride and sulfate, indicating that these anions interfere strongly with carboxylic acid extraction with amine extractants.

The extraction (K_D) was found to be greatly affected by the amine content in the extractant, the solution pH, the concentration of acids, and the presence of other anions. In general, adding a polar

diluent (e.g., 2-octanol) to the amine extractant (Adogen 283 or Alamin 336) increased the extraction of acetic acid and lactic acid greatly; however, the effects were quite complicated and not linear. More details on the extraction chemistry, which involves ion complexation reaction, and extraction mechansim in single acid and multiple acid mixture systems can be found elsewhere (Jin, 1997; Jin and Yang, 1998; King, 1992; Qin and Yang, 1997).

Table 4.1. Distribution Coefficients (K_D) for Various Amine Extractants*

Aqueous Sample	Alamine 336	Aliquat 336	Adogen 283	Amberlite L-1
4% Acetic acid				
in water	2.8	0.9	16.1	4.9
in whey	2.7	-	9.0	-
in fermentation broth#	0.8	-	1.0	-
in 0.66M KCl soln.	0.8	-	1.0	-
In 0.33M Na_2SO_4 soln.	0.9	-	1.2	-
4% Lactic acid				
in water	4.9	0.5	8.4	2.4
in whey	5.0	-	-	-
in fermentation broth#	0.5	-	0.9	-
in 0.66M KCl soln.	0.3	-	-	-
In 0.33M Na_2SO_4 soln.	0.5	-	0.5	-

*50% amine in 2-octanol
#NaOH was added during fermentation and the broth was acidified to ~pH 2 with H_2SO_4.

4.2 CONTINUOUS EXTRACTION (PACKED-COLUMN EXTRACTOR)

4.2.1 Extraction

Continuous extraction experiments were conducted to study extraction of acetic acid. A packed bed column extractor (diameter: 2.2 cm, length: 53 cm, packing: 0.635 cm ceramic saddles, void volume: ~140 mL) was used. The experiment was conducted with an extractant consisting of 50% Alamine 336 in 2-octanol. The acetic acid solution and the solvent were fed counter-currently through the extractor. The bottom half of the column was filled with the aqueous phase and the upper half with the organic phase. The phase separation line was maintained at the mid-plane of the column throughout the extraction experiment. The extractant containing acetic acid was collected at its outlet from the extractor and was then fed to another extractor where a 6*N* NaOH solution was used to back-extract acetic acid from the extractant.

Two different concentrations of acetic acid solutions were used to study the extraction performance of the continuous packed column extractor. The acetic acid concentrations in the aqueous effluents from the extractor operated at various retention times are shown in Figure 4.1. When the feeding acetic acid concentration was 13.3 g/L, it required 3 h retention time to remove 90% acetic acid from the feed solution to the solvent phase. With 24.25 g/L acetic acid in the feed solution, 4.68 h

was required to attain 90% extraction. However, most extraction was achieved with short retention times (less than 2 h). When the retention time was longer than 2 h, extraction efficiency was not high. It is noted here, however, that the extractor design has not been optimized. The purpose of this experiment was just to demonstrate that low concentrations of acetic acid could be efficiently extracted with Alamine 336 or Adogen 283.

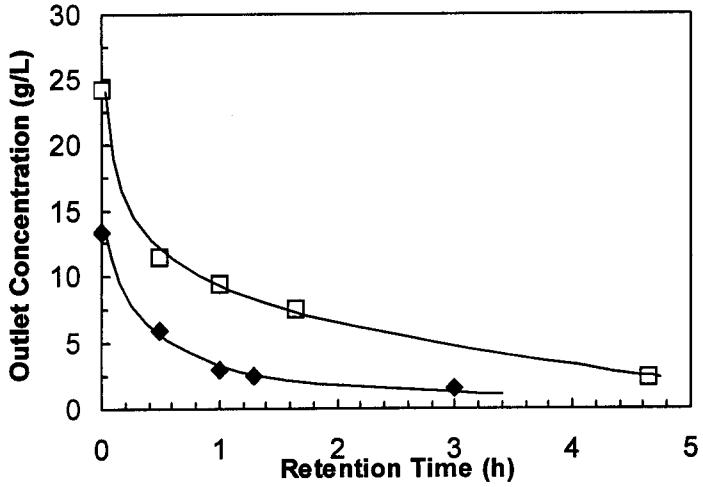


Figure 4.1. Performance of Continuous Extraction of Acetic Acid with Alamine 336 in a Packed-Column Extractor. (Feeding concentration (g/L): ◆- 13.3; □ - 24.25)

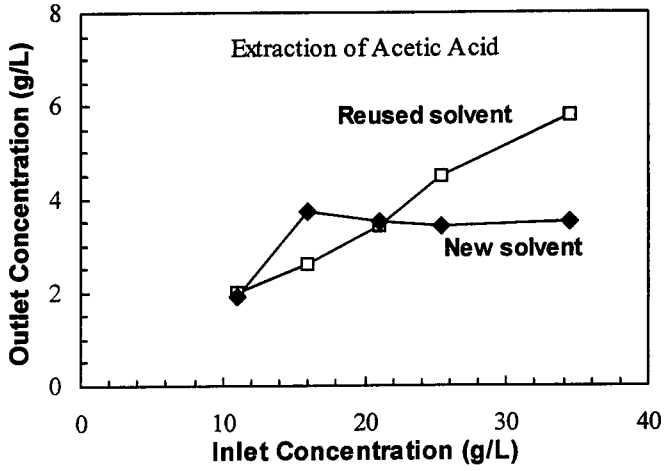


Figure 4.2. Comparison of Extraction Performance Between New and Regenerated Solvents. (□-Reused Solvent; ◆-New Solvent)

4.2.2. Solvent Regeneration

The acetic acid present in the extractant can be back-extracted with an alkali solution, thus allowing the extractant to be regenerated and reused. Experiments were conducted to study the efficiency of

back-extraction and the performance of regenerated solvent in extraction. Used Alamine 336 was regenerated by mixing with 1 N NaOH. The regenerated solvent was then used in continuous extraction and the results are compared with those from new solvent. In this experiment, various feed acetic acid concentrations were used. The retention time was fixed at ~2 h. The outlet acetic acid concentrations were measured and are shown in Figure 4.2.

As shown in Figure 4.2, the regenerated solvent had comparable extraction performance to the new solvent. When the inlet acetic acid concentration was 20.8 g/L, the acetic acid concentration of both new and regenerated solvent were almost the same. At a higher feed acetic acid concentration, the performance of the regenerated solvent was only slightly worse than that of the new solvent. At feed concentration of 32.14 g/L, the outlet concentration of used and new solvent was 6.53 and 5.79 g/L, respectively. These results indicated that back-extraction with alkali solution was able to regenerate the solvent and to allow the solvent to be used repeatedly.

4.2.3 Continuous Extraction and Back-extraction

The continuous two-step extraction process was demonstrated using two packed-column extractors, shown in Figure 4.3. The extraction column was 5 cm x 70 cm and had 700 mL void volume after packing. The back extraction column was 4 cm x 50 cm, with 400 mL void volume. Unless otherwise noted, the feed stream was 4% acetic acid in water, the extractant was 50% Alamine 336 in 2-octanol, and the base used in back-extraction was 6N NaOH. The extractant was recirculated between the two columns for extraction and back-extraction, respectively. The lactic acid concentrations in the various streams were monitored to evaluate process performance.

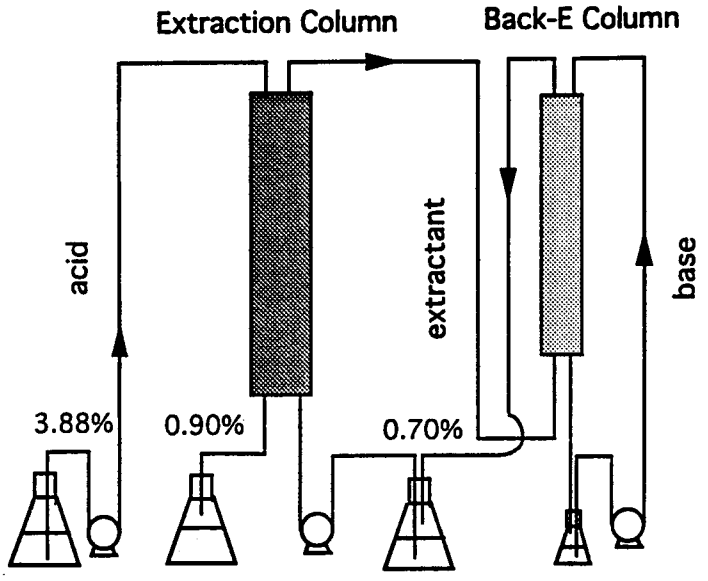


Figure 4.3. Experimental Set-Up for Continuous Extraction and Back-Extraction.

Extraction of acetic acid in water with 50% Alamine 336 in 2-octanol and back-extraction with 6N NaOH solution using the two-column system was studied. The acetic acid concentrations in the two aqueous outlet streams (feed and base) were monitored. As shown in Figure 4.4, about 60% of the

acetic acid present in the feed stream was extracted into the solvent and all acetic acid in the solvent was back-extracted into the base stream. The process reached steady state within several hours. A higher recovery (>95%) can be achieved with different operation conditions. This experiment demonstrated that solvent can be regenerated continuously in the stripping column and reused efficiently in this two-step extraction process.

The two-step extraction process was then demonstrated with lactic acid using the same two continuous, packed-column extractors. The feed stream was 5% lactic acid in water, the extractant was 50% Alamine 336 in 2-octanol, and the base used in back-extraction was 6N NaOH. Depending on the operating conditions, from 60% to 90% of the lactic acid in the feed stream was extracted by the solvent and stripped by the base solution. In this experiment, the base stream was recycled to observe the increase of lactate concentration in the base stream (stripping or product stream). The purpose of this experiment was to demonstrate that dilute acid can be recovered and concentrated efficiently using the present system.

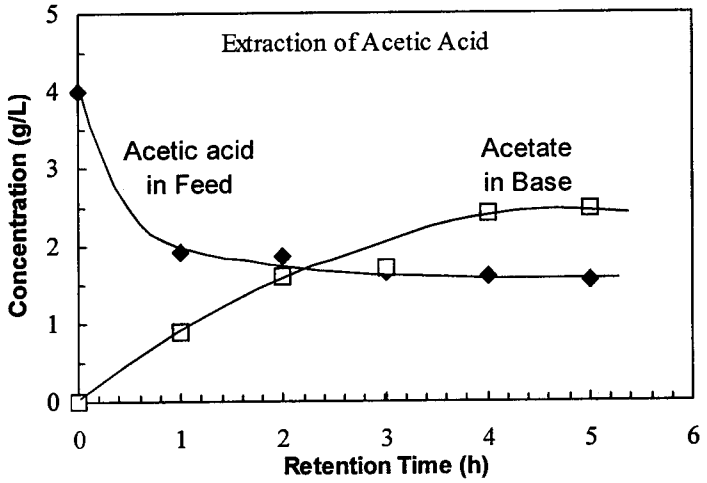


Figure 4.4. Outlet Concentrations of Acetic Acid From Feed and Stripping (Base) Solutions From the Continuous Extraction and Back-Extraction Process.

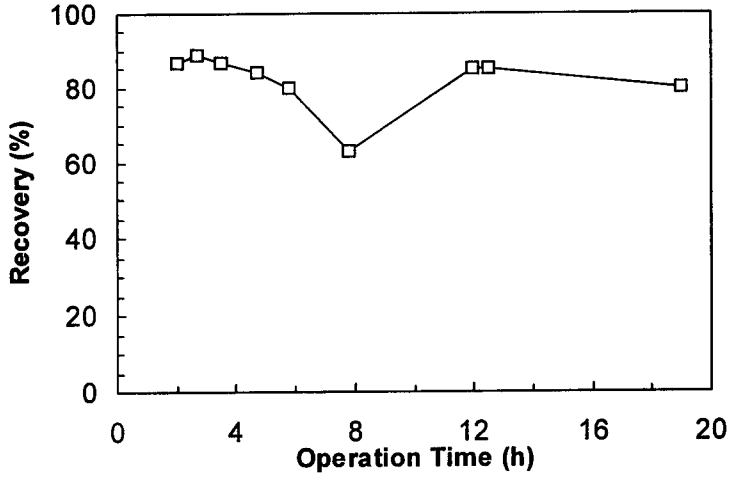


Figure 4.5. Performance of Lactic Acid Recovery Using the Two-Step Extraction Process.

As shown in Figure 4.5, the recovery was ~85%, with some fluctuations due to changes in pump speed and feed rate. The significant drop at ~8 h was due to failure in maintaining an appropriate phase separation line in the column extractor during the experiment. Once the problem was corrected, the extraction performance returned to normal condition. The lactate concentration in the base (product) stream increased to ~20% (w/v) at a constant rate with time (Figure 4.6), indicating that this two-step extraction process is very stable. Further experiment showed that sodium lactate concentration reached a maximum of 70% (w/v) (Figure 4.7). Thus, the process was proved to be feasible to recover and concentrate organic acids from dilute solutions. It is conceivable that 50% potassium acetate solution can be economically produced from 4% acetate broth using this two-step extraction process. Back-extraction with dolime or dolomitic limestone for CMA production presents a different and more difficult operation problem as compared with NaOH or KOH, and will be discussed later.

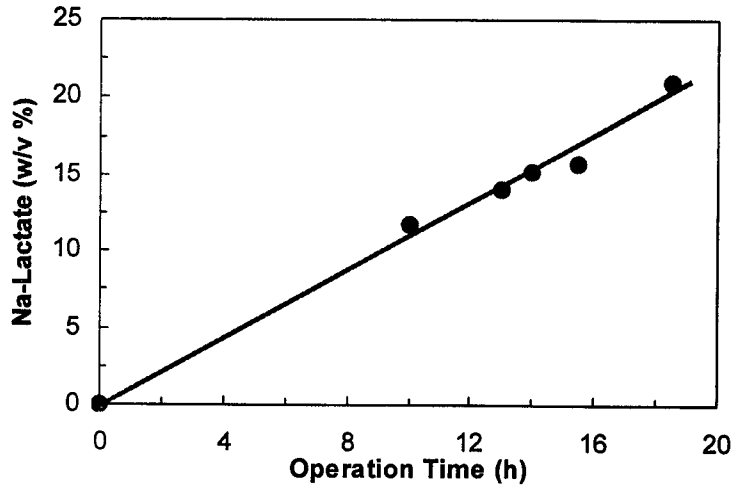


Figure 4.6. Change of Lactate Concentration in the Stripping (Base) Stream with Time.

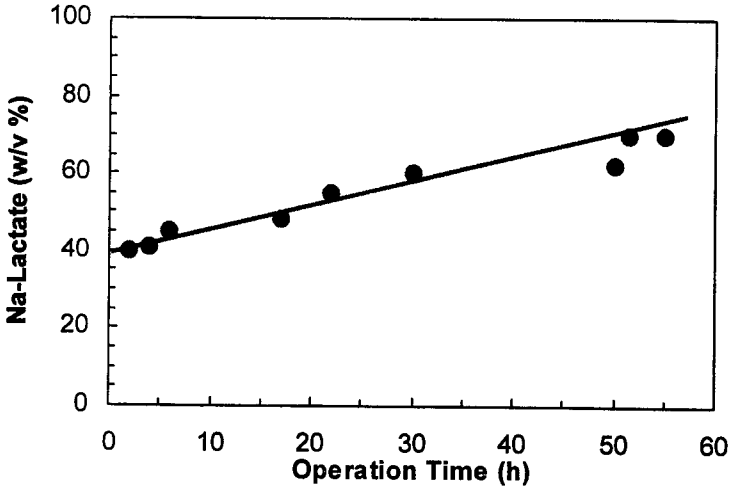


Figure 4.7. Lactate Concentration in the Stripping Stream Increased to 70% (w/v).

Table 4.2 summarizes some of the experimental results obtained from the two-step extraction process. In general, a higher organic phase feed rate can be used to improve the extraction efficiency.

Table 4.2. Performance of the Two-Step Extraction Process for Organic Acid Recovery.

Feed/Solvent	V_{aq} (ml/min)	V_{org} (ml/min)	Recovery (%)	K_D	k_ma (min^{-1})
5% lactic acid in water/Alamine 336	1.5 3.0 1.5	4.5 3.0 3.0	85 66 75	5 5 5	0.002333 0.002687 0.001682
4% lactic acid in broth/Alamine 336	1.5	4.5	50	0.5	0.001673
4% acetic acid in water/Alamine 336	3.0	3.0	60	2.5	0.002574
4% acetic acid in broth/Adogen 283	1.5	4.5	55	1.0	0.001237

4.2.4 Effects of Anions on Extraction

When fermentation broth was used as the feed to the extraction process, its pH has to be adjusted to below pK_a to facilitate the acid extraction. However, the broth acidified with either sulfuric acid or HCl yielded low extraction efficiency, only ~50% of that found with water (containing organic acids) as the feed. At about the same operating conditions, acid recovery was 40% - 50% with acidified broth, compared to 70% - 90% with water.

The reduced extraction performance was due to the reduced K_D value for carboxylic acid in the presence of sulfate or chloride ions (see Table 4.1), which competes strongly for the active sites of the amine extractant. These anions also seemed to be stripped into the base solution, thus contaminating the product stream. This problem could be attributed to the sulfuric acid used to acidify the fermentation broth before extraction. The problem thus may be alleviated by limiting the sulfate concentration in the broth. One possible solution is to use lime (CaO), instead of NaOH or NH_3, to control the fermentation pH. The calcium ion will react with sulfate ion to form $CaSO_4$ precipitate upon acidification with sulfuric acid. Alternatively, a weak acid such as carbonic acid or CO_2, instead of a strong acid, may be used to acidify the broth.

4.2.5 Gas Stripping for Acetic Acid Recovery

As discussed earlier, acidification of the fermentation broth is necessary for efficient extraction of acetic acid. However, the addition of a strong acid may interfere with acetic acid extraction, lower the extraction efficiency, and may even contaminate the final acetate product if not handled properly (see the next section on Sample Preparation). Steam or air stripping is feasible but also requires acidification. The feasibility of acidifying the fermentation broth with CO_2 gas to facilitate acetic

acid extraction was thus investigated. Sodium acetate solution (pH 9.0) was mixed with Adogen and sparged with CO_2 gas at ambient pressure. Acetic acid concentrations in the aqueous and solvent phases were monitored. It was found that significant amounts of acetic acid were transferred to the solvent phase in this three-phase, extraction/stripping system. Thus, it is possible to extract acetic acid from the fermentation broth directly without adding sulfuric acid. However, more work is necessary.

4.2.6 Phase Separation

In the above experiments, some aqueous phase (stripping fluid) containing the organic salt was carried out as emulsion with the organic phase, thus reducing the extraction efficiency. This emulsion can be better controlled at a pH around 9 and at a lower base flow rate. This two-step extraction process can be further improved if better phase separation in the back-extraction column can be achieved. A non-polar diluent, such as hexadecane, which is more hydrophobic than the polar diluent (2-octanol), may be used to facilitate phase separation. However, test tube phase separation experiments showed that there was no significant difference in phase separation time for various solvents (Alamine/2-octanol, Alamine/hexadecane, Adogen/2-octanol, Adogen/hexadecane) and aqueous phases (4% acetic acid solution, 1*N* NaOH solution). For all two-phase systems studied, the two phases separated in ~30 minutes after vigorously mixing with Vortex for 1 minute.

A hollow-fiber membrane extractor can be used to avoid a phase separation problem (if it is a problem), and was studied later for its use in extractive fermentation.

4.2.7 Mass Transfer Coefficient

The mass transfer coefficient, $k_m a$, in the packed bed extractor was evaluated using the mathematical model for a differential extractor (see equations given in the extractor design section). The values for $k_m a$ under various flow conditions are also shown in Table 4.2. In general, $k_m a$ increases with increasing the superficial velocity in the packed column, following the correlation shown below:

$$k_m a = 0.04272\, u_{aq}^{0.676}\, u_{org}^{0.807} \qquad (4.1)$$

where $k_m a$ is in min^{-1}, the superficial velocities u_{aq} and u_{org} are in cm/min.

4.2.8 Effects of K_D and $k_m a$ on Extraction

The extraction efficiency is sensitive to K_D and $k_m a$, but the effects diminish as K_D is greater than 2 and $k_m a$ is greater than 0.006 min^{-1}. The effect of K_D also diminishes as the ratio V_{org}/V_{aq} increases. In general, when K_D is greater than 3, mass transfer becomes the limiting step in the packed column extractor.

4.2.9 Effects of V_{org}/V_{aq} on Extraction

In general, increasing the V_{org}/V_{aq} ratio would improve the extraction when extraction is limited by the extractant capacity or K_D is low and $k_m a$ is moderately high. On the other hand, the effect is not significant when extraction is limited by mass transfer or $k_m a$ is low and K_D is moderately high.

4.3 BACK-EXTRACTION WITH DOLIME

Back-extraction with dolime or dolomitic limestone is a more complicated operation than with NaOH or KOH, because of the involvement of a third, solid phase (the solubilities of CaO, $CaCO_3$, MgO, and $MgCO_3$ are very low in water). At low concentrations (for up to ~10% acetate concentration), the back-extraction process worked as well as expected when the packed column apparatus was used. However, to push for higher acetate concentrations (e.g., ~25%) in the stripping fluid, more concentrated dolime slurry needs to be used but it is very difficult to operate with the packed column under this condition. The presence of high solid contents not only causes solid deposits in the packed column but also prevents good contact between the two liquid phases.

To eliminate the solid phase, an alternative process using $CaCl_2$ and $MgCl_2$ as the stripping chemicals for CMA formation is shown in Figure 4.8. This process, however, probably would not be economical for CMA production due to its complexity and high material costs.

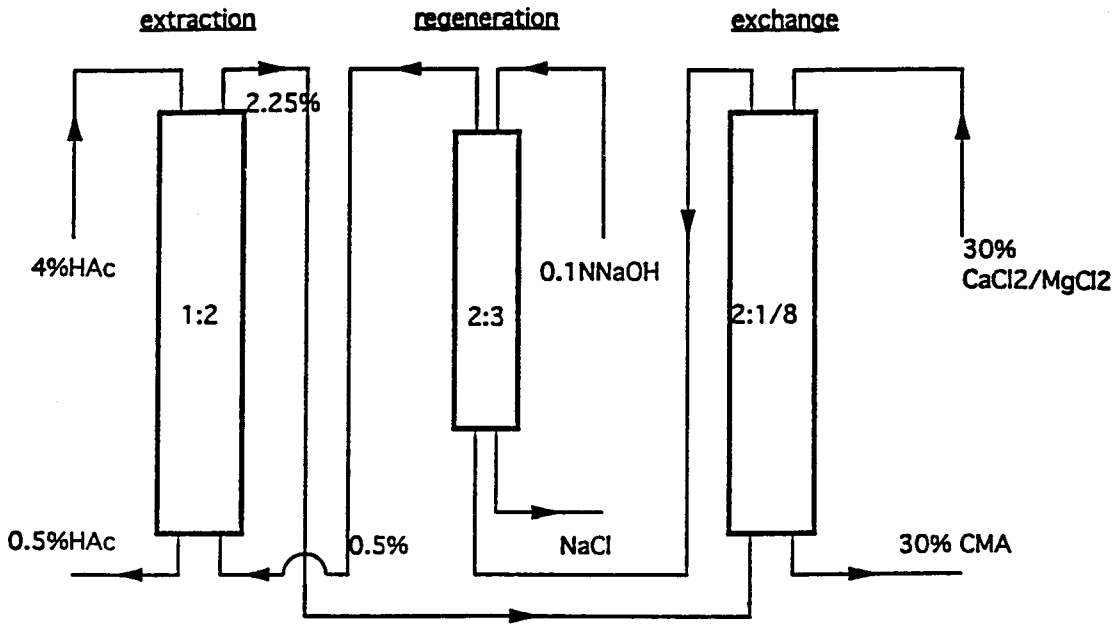


Figure 4.8. An Alternative Three-Step Extraction Process for CMA Production.

Back-extraction with the packed column was thus abandoned (this has never been intended for the real process operation, anyway). Back-extraction with CaO slurry in flasks with vigorous mixing, followed with centrifugal separation of solvent, water, and solid, was conducted. One volume of the CaO slurry (at ~10% wt/v) was mixed with two volumes of the extractant (50% Adogen 283 in 2-octanol) containing 4.5% acetic acid. After each stripping contact and centrifugal separation, the resulting calcium acetate solution was replenished with CaO and then used again in back-extraction. The results are summarized in Table 4.3.

After three stripping contacts, 20.35% acetic acid (or equivalent to 27% CMA) was obtained in the stripping fluid (CaO slurry). More than 99% of acetic acid present in the solvent phase was stripped in the first two contacts. However, the stripping efficiency decreased significantly to ~83% in the

3rd contact where the equilibrium acetate concentration was high (20.35%). This experiment indicates that CaO or dolomitic lime slurry can be used to strip and react with acetic acid in the extractant, but the efficiency would be lower at higher acetate concentrations. This problem may be solved by intelligent process design. Thus, the proposed back-extraction process also should work well with dolime or dolomitic limestone.

Table 4.3. Results from Back-Extraction of Adogen Extractant with CaO Slurry.

Contact #	Acetic acid in water (%)		Acetic acid in solvent (%)		Material Balance
	before	After	before	after	
1	0	8.83	4.5	0.02	79%
2	8.83	15.86	4.5	0.04	79%
3	15.86	20.35	4.5	0.78	67%

The material balance in the back-extraction experiment shows that significant amounts (more than 20%) of acetic acid were not recovered. The possibility of CMA adsorption by CaO particles has been ruled out based on adsorption experiments. It is not clear at this point if the missing acetate was due to experimental errors or reaction with the amine extractant (Adogen 283 contains 10%-20% primary amine and alcohol).

A similar back-extraction experiment was conducted with Alamine 336. The results are summarized in Table 4.4. The back-extraction efficiency was only ~75% in this experiment due to the short contact time allowed for the three phases (they were centrifuged and separated after only several minutes contact). Otherwise, the results are similar to the previous experiment with Adogen 283. The material balance was also lower than 100%. Further experiments should be conducted with larger volumes to minimize material loss during transfer and possible volume changes after phase contacts.

Table 4.4. Results from Back-Extraction of Alamine Extractant with CaO Slurry.

Contact #	Acetic acid in water (%)		Acetic acid in solvent (%)		Material Balance
	before	After	before	after	
1	0	4.82	4.5	1.14	77%
2	4.82	10.18	4.5	1.17	89%

4.3.1 Conclusion and Summary

The two-step extraction process is energy-efficient in recovering and concentrating carboxylic acids from dilute fermentation broth. Amine extractants with K_D greater than 2 will be appropriate to use in this process. The process would be more efficient if higher mass transfer coefficients can be

achieved in the differential extractor. It is also necessary to improve phase separation properties of the extractant, especially during back-extraction with base. Also, it is desirable to improve the extractant performance at pH values much higher than pK_a value of the acid or close to the optimal fermentation pH, which is usually close to 7. This also will allow extractive fermentation, in which the organic acid is extracted from the broth as it is formed, to be conducted more efficiently. This would eliminate the addition of base during fermentation. Back-extraction of the amine solvent containing acetic acid with lime or dolime yields a concentated CMA solution that can be economically dried without further concentration by evaporation.

4.4 EXTRACTION USING HOLLOW-FIBER MEMBRANE EXTRACTORS

Hollow-fiber membrane extraction combines extraction with membrane processes developed during the past 10 years. It is attractive because it makes the extraction process less dependent on the physical properties of solvent, reduces the entrainment of solvent, increases mass transfer area, and reduces emulsification. It also greatly reduces toxic effects of the organic solvents on the microorganism in an extractive fermentation (Solichien et al., 1995). Since Kiani et al. proposed the hollow-fiber membrane extractor in 1984, some research has been carried out in separating carboxylic acids in aqueous solutions, including citric acid (Basu and Sirkar, 1991, 1992; Juang and Chen, 1996), acetic acid (Sengupta et al., 1988), lactic acid (Chen and Lee, 1997), and propionic acid (Solichien et al., 1995; Jin and Yang, 1998). However, very little has been on separating a carboxylic acid from a mixture of carboxylic acids present in the solution. Most of the prior work concentrated on the effects of membrane material and organic solvent on the extraction efficiency, and mass transfer resistance in the transport process.

The objective of this study was to develop an extraction process suitable for use in extractive fermentation. Unlike conventional extraction, which is mainly concerned about having a high extraction recovery, it is important to have a high extraction rate in extractive fermentation. It is also important to have a high selectivity in a multi-component extraction system. Therefore, for this work, the objectives were to find conditions that would give both a high extraction rate and high selectivity for acetic acid since lactic acid was also present in the solution. Membrane resistance is an additional barrier for the mass transfer process in a membrane extractor, which may reduce the overall mass transfer coefficient. In a multiple component system, the mass transfer coefficient for different species (lactic and acetic acids) would be different because their molecular diffusion coefficients in water are different (they have different molecular weights and sizes). Both the mass transfer coefficients and the kinetic parameter, e.g., distribution coefficient, K_d would affect the extraction rate and selectivity in the extraction process.

In this work, the extraction of acetic acid from a solution containing the mixture of lactic acid and acetic acid was studied in a set of hollow-fiber membrane extractors. The extraction characteristics of a single component system (acetic acid only) is compared with that of the mixture system. Water-acetic acid, lactic acid-Adogen 283 (2-octanol) was used as the experimental system. Adogen 283 (2-octanol) was the extractant. The effects of the amine content in the solvent phase, the solution pH, initial acid concentrations, and the ratio of acetic acid to lactic acid in the aqueous solution on the extraction rate and selectivity were studied. The effects of the flow rate of each phase and the initial acid concentration on the extraction rate for

acetic acid in HFME were also studied. The mass transfer coefficients for acetic acid and lactic acid in HFME were calculated based on a plug-flow model. The extraction mechanism of the acetic/lactic acid mixture is also discussed. The potential application of this extraction process for an extractive fermentation process to produce acetate was also investigated.

4.4.1 Experimental Set-Up

Figure 4.9 shows the hollow-fiber membrane extraction system used in this study. The HFME (Celgard X-30, Hoechst Celanese Corporation) contained hydrophobic, isotactic polypropylene hollow fibers (240 μm I.D.; 30 μm wall thickness; 300 μm O.D; membrane porosity: 40%). Adogen 283 was the extractant and 2-octanol was used as the diluent. The solvent (organic phase) flowed in the tube-side and the feed (aqueous phase) flowed in the same direction in the shell-side in HFME. Inside the shell of the HFME, the aqueous phase actually flowed over the surfaces of the hollow fibers. To feed both phases in the same direction gave an evenly distributed pressure gradient between the two sides of the hollow fiber membrane and along the entire extractor length, which allowed an easier operation without significant pressure fluctuation and breakthrough of either phase through the membrane. The stripping solution used in the back-extraction step was 6N NaOH in distilled water, which also flowed in the shell-side in the same flow direction as the organic phase. Aqueous and solvent samples from both inlets and outlets of the tube and shell sides were taken when each phase had run for six to eight volumes of the HFME to ensure reaching steady state. Total material balance was used to confirm the system was at steady state. The effects of various operation parameters on the extraction behavior were studied, including flow rates of aqueous phase (V_a), organic phase (V_o) and stripping solution (V_s), the amine content in the organic solvent, initial acid concentration in the aqueous phase (X_o), pH of the aqueous phase, and the ratio of acetic acid to lactic acid (R).

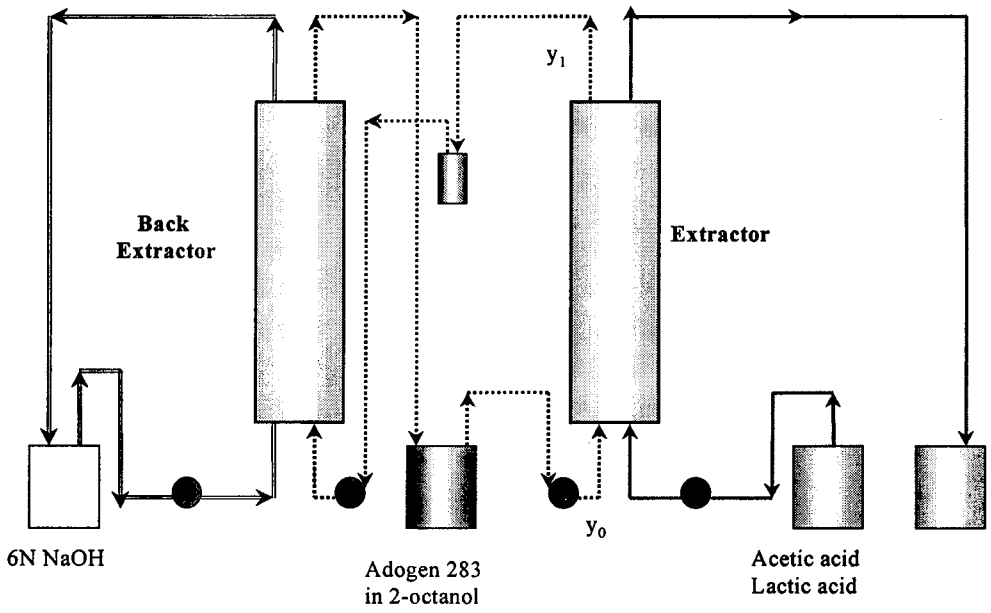


Figure 4.9. A Hollow-Fiber Membrane Extraction System for Separating Acetic Acid from the Feed Solution.

4.4.2 Effects of Flow Rate and pH

Figure 4.10 shows the effects of flow rate and solution pH on the extraction rate of acetic acid in the single component system (containing only acetic acid). In general, increasing the flow rates increased the extraction rate, because of improved mass transfer through the membrane. However, the effect was not as significant at pH 5.5 as at pH 5.0. The extraction rate was also higher at pH 5.0 than at pH 5.5. Increasing the flow rate of the stripping solution would have a positive effect on solvent regeneration and lowered the value of y_1, thereby allowing more acetic acid to be extracted by the solvent. This experiment also indicated that the overall extraction rate in this two-step extraction process depended on both the extractor and back-extractor.

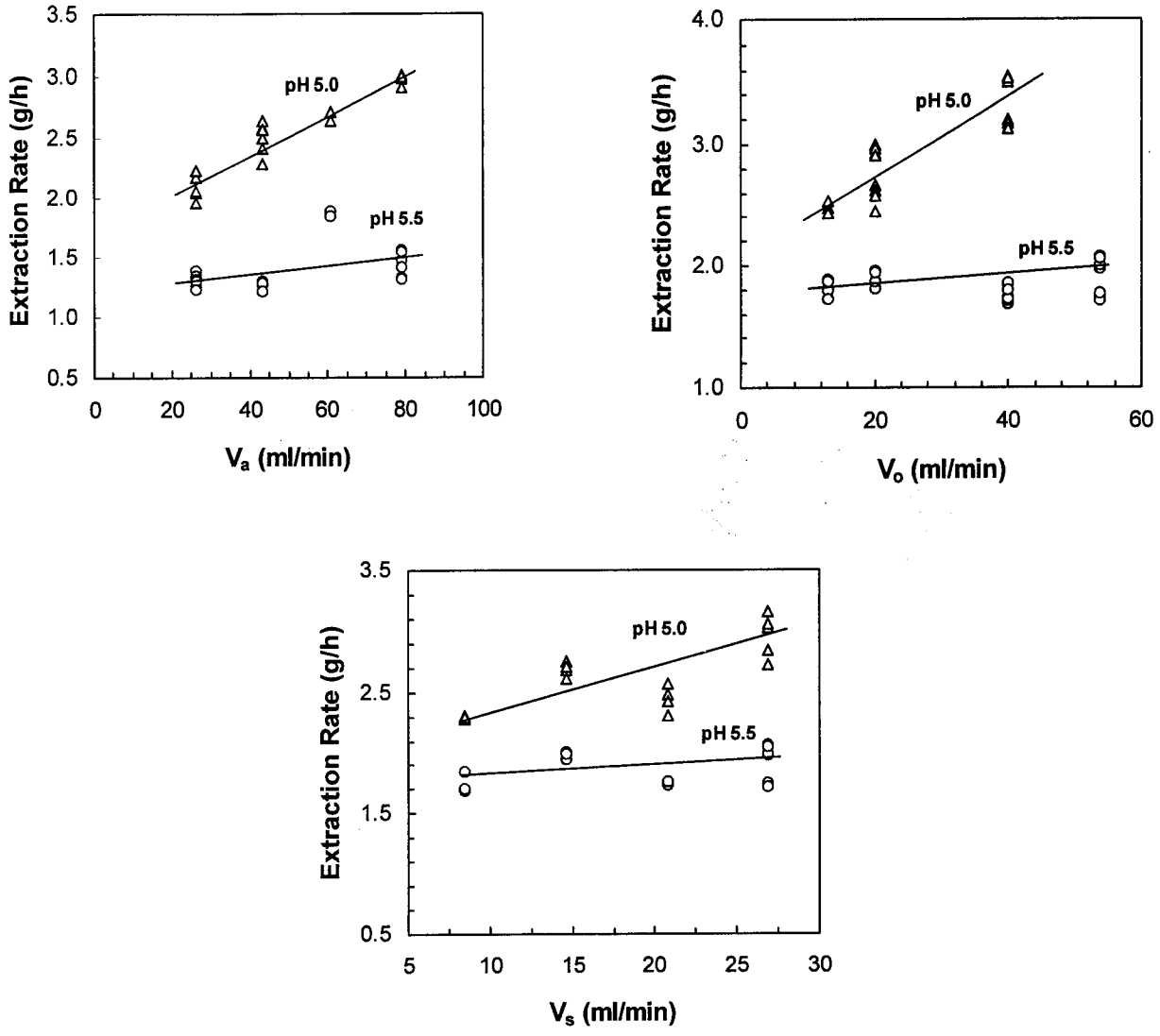


Figure 4.10. Effects of Flow Rate of Aqueous Phase (V_a), Organic Phase (V_o), and Stripping Solution (V_s) on the Extraction Rate at pH 5.0 and pH 5.5.

4.4.3 Effects of Concentration

The extraction rate also increased with increasing the concentration of amine in the solvent and acid concentration in the aqueous solution for both acetic acid and lactic acid (Figure 4.11). This is because increasing amine content would increase K_d value, and increasing acid concentration would increase the mass transfer driving force, concentration gradient, between the two phases. It is noted that the K_d value for acetic acid was lower at pH 5.0 and higher at pH 5.5 than that for lactic acid. This suggests the use of pH 5.5 for a better selectivity for acetic acid over lactic acid in a mixture system.

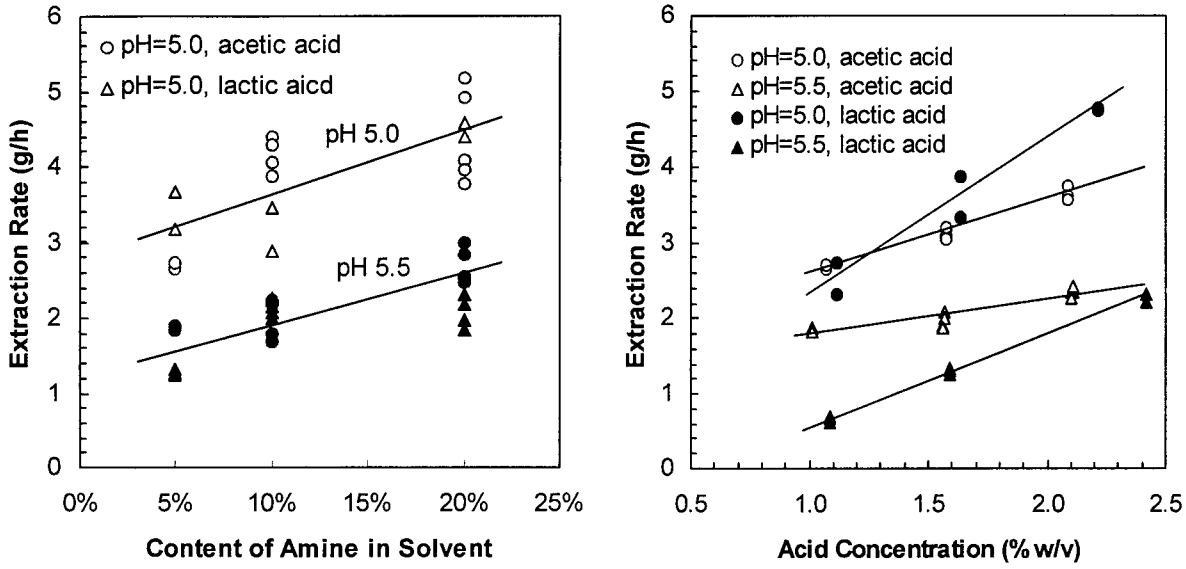


Figure 4.11 Effects of Amine Content in Solvent and Acid Concentration in the Aqueous Phase on the Extraction Rate.

4.4.4 Selectivity

The effects of acid concentration ratio (acetic acid to lactic acid ratio) and amine concentration on the extraction selectivity for an acetic/lactic acid mixture system are shown in Figure 4.12. Again, the selectivity was better at the higher pH of 5.5. The overall mass transfer coefficients, K_o, for acetic acid and lactic acid in the system were estimated and the results are shown in Figure 4.13. Increasing the amine content in the solvent also increased the viscosity and thus decreased K_o.

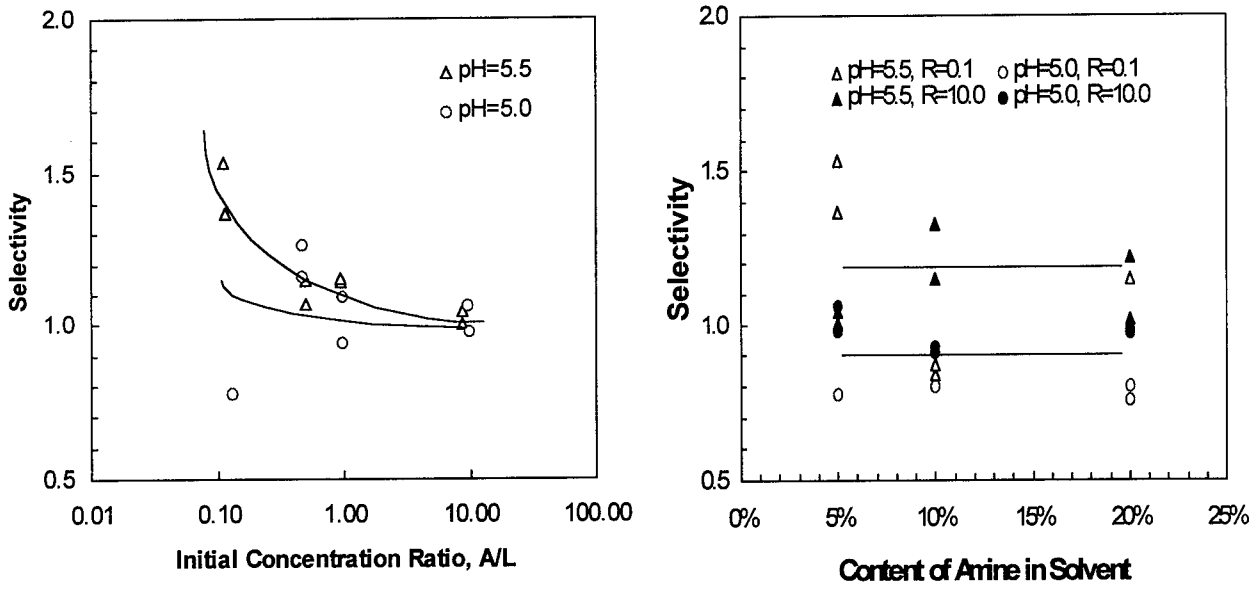


Figure 4.12. Selectivity as Affected by the Acetic Acid to Lactic Acid Concentration Ratio (A/L) and Amine Content in Solvent.

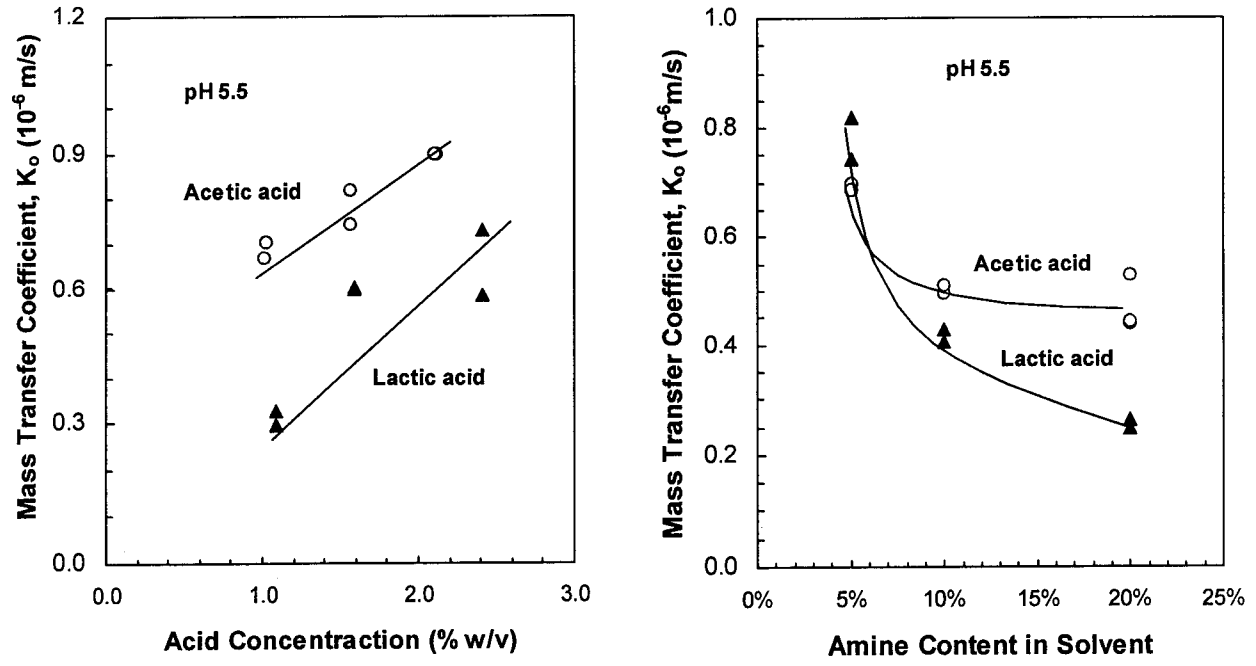


Figure 4.13. Overall Mass Transfer Coefficient as Affected by the Acid Concentration Ratio and Amine Content in Solvent.

4.4.5 Simulated Extractive Fermentation

Simulation experiments were conducted to investigate the feasibility of the HFME system for separating acetic acid from the fermentation broth in an extractive fermentation. The feeding tank (as a fermentor) initially contained a solution (pH 5.5) containing the mixture of acetic acid and lactic acid at different concentrations. As extraction took place, lactic acid was added to the feeding tank to maintain the solution pH at 5.5. The addition of lactic acid would acidify acetate ions in the solution and thus allowed the extraction of acetic acid by the solvent. As shown in Figure 4.14, only acetic acid was extracted initially (for the first few hours) when lactic acid concentration in the solution was low. However, as the lactic acid concentration continued to increase and acetic acid concentration decreased, more lactic acid and less acetic acid were extracted, as indicated by the concentrations of lactate and acetate present in the stripping solution.

Another experiment was conducted to better simulate the extractive fermentation, where the acetic and lactic acid concentrations in the solution (fermentation broth) were maintained at a relatively constant level by feeding the same composition of mixture solution to the tank as extraction proceeded. As shown in Figure 4.15, mainly acetic acid was extracted in this system. This experiment suggested that in extractive fermentation, lactic acid concentration should be maintained at a low level and acetic acid concentration should be much higher than that of lactic acid in order to facilitate the extraction of acetic acid. A high purity of acetate product can be obtained in the extractive fermentation.

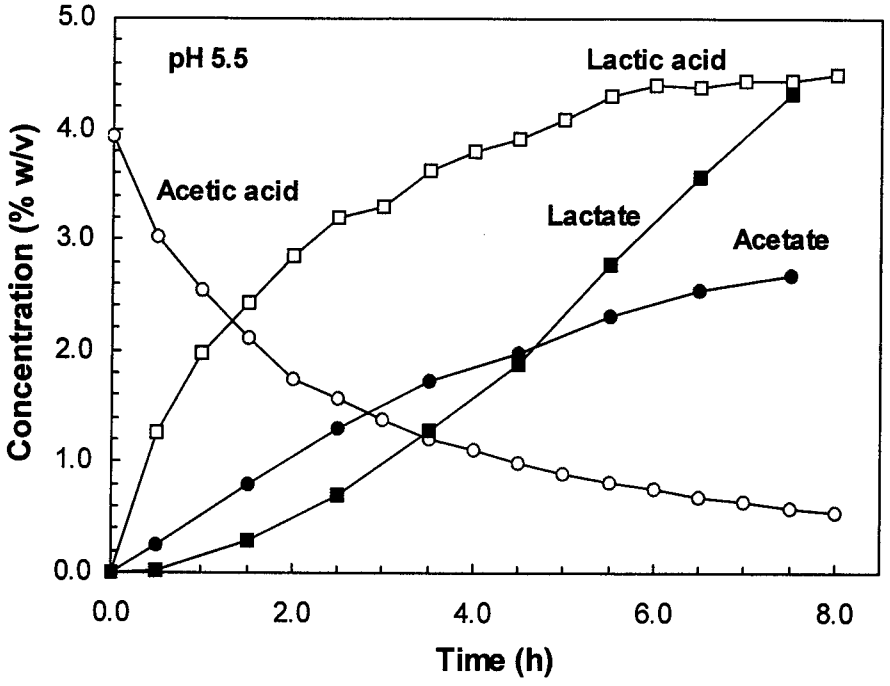


Figure 4.14. Continuous Extraction of Acetic Acid From Aqueous Solution at pH 5.5 by Adding Lactic Acid (15% w/v Solution).

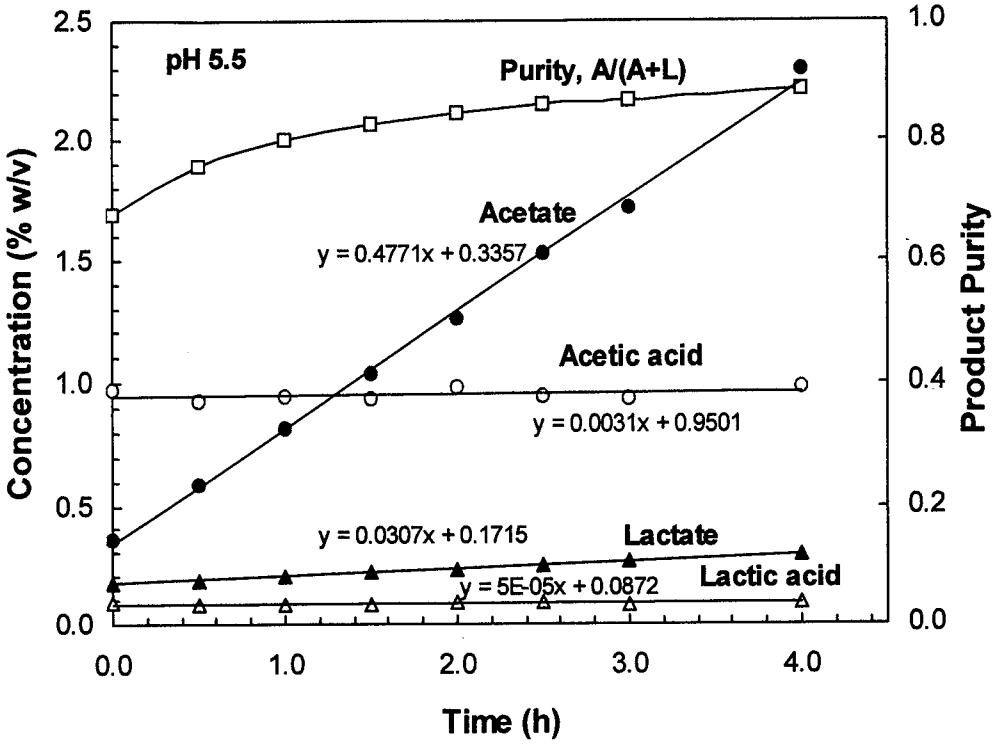


Figure 4.15. A Simulated Extractive Fermentation - Continuous Extraction of Acetic Acid From the Solution Containing 1% Acetic Acid and 0.1% Lactic Acid at pH 5.5 by Continuously Adding the Solution to Maintain a Constant pH 5.5.

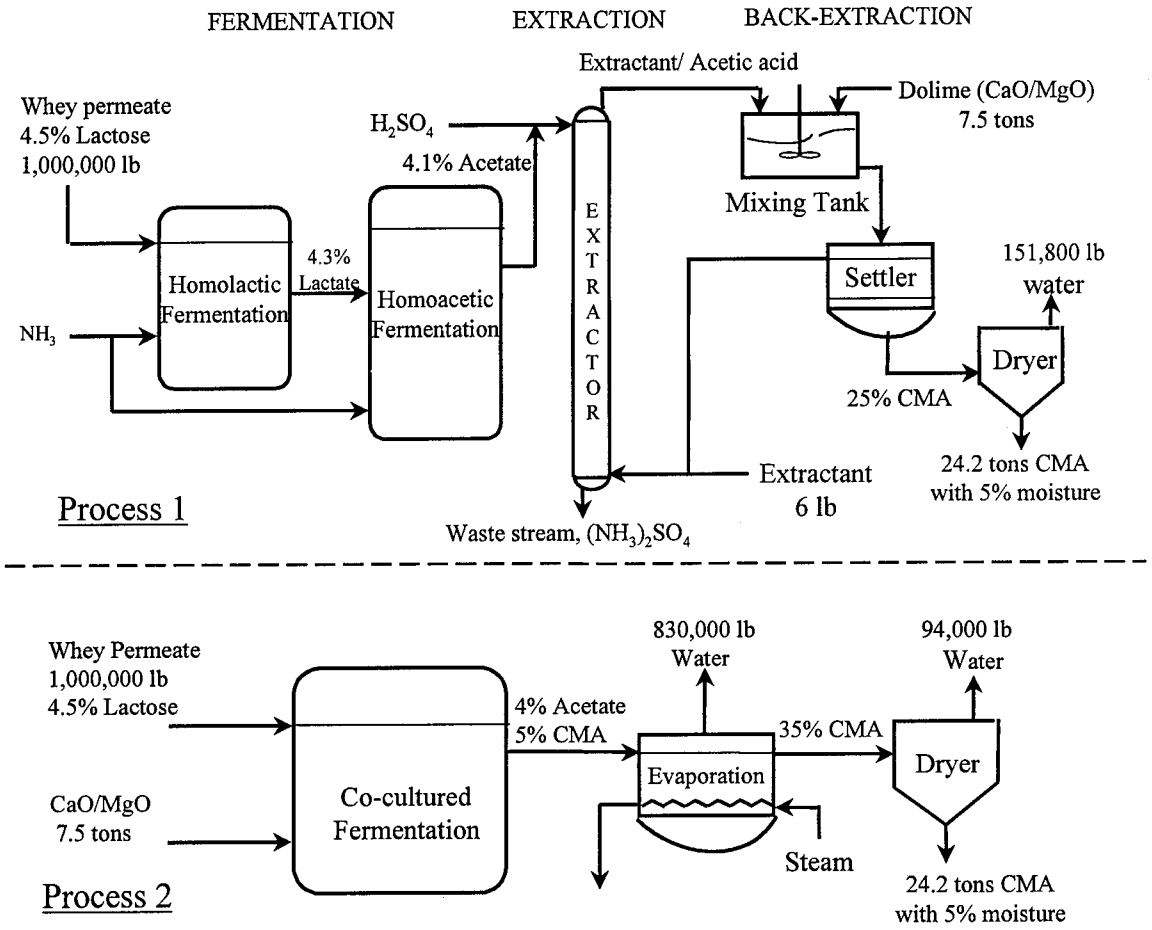
4.4.6 Conclusion and Summary

It is concluded that acetic acid can be effectively extracted and separated from lactic acid using 5% Adogen 283 in 2-octanol in the HFME. The extraction rate was higher at a lower solution pH, but selectivity was better at pH 5.5. A higher initial acid concentration, higher acetic acid to lactic acid ratio, and higher flow rates also are desirable for increased extraction rate and selectivity. These operating conditions are thus recommended for use in an extractive fermentation for acetate production.

V. CMA PRODUCTION PROCESS AND SAMPLE TESTING

5.1 CMA PRODUCTION PROCESS

Two potential processes to produce acetate and CMA from cheese whey based on the fermentation and extraction technologies discussed before are shown in Figure 5.1. The technical feasibility of the fermentation and extraction to be used in the processes has been discussed in the previous sections. Several CMA samples were prepared from whey following the process steps shown in Figure 1.1. They were then tested for their likeness to the present commercial CMA in their chemical composition and deicing performance. The purpose of this work was to evaluate the qualities and deicing ability of the CMA produced from cheese whey.



2.2 lb = 1 kg; 2200 lb = 1 tonne; 1.1 tons = 1 tonne

Figure 5.1. Two CMA Production Processes – Process 1 Uses Fermentation to Produce Acetate and Extraction to Recover and to Produce CMA; Process 2 Produces a Crude CMA from the Whole Fermentation Broth.

5.2 CMA SAMPLE PREPARATION

5.2.1 CMA Samples from Process 1

The first sample (Sample #1) was prepared as follows: 3 liters synthetic media containing 4% lactate were fermented in a batch fermentor (37°C, pH 7.6, with NaOH) for about 5 days. The broth containing ~3.5% acetate was acidified to pH 3 with sulfuric acid, extracted with an amine extractant, and then back-extracted with CaO-MgO slurry. The CMA-containing solution was then dried and the solids were crushed to powder.

A second sample (Sample #2) was prepared from acid whey in a similar way. The acid whey was first fermented in a homolactic bioreactor to convert lactose to lactate. The broth was then added with some yeast extract and trypticase, autoclaved, and then fermented again in a homoacetic bioreactor to convert lactate to acetate. The final broth containing sodium acetate was then acidified with sulfuric acid, extracted with the amine extractant, and back-extracted with dolomitic limestone ($CaCO_3$-$MgCO_3$) slurry. The three phases, organic, aqueous, and solid (unreacted dolime), were then separated by centrifugation. The aqueous phase containing CMA was dried and crushed to obtain the powder product.

It was found later that these two CMA samples contained significant amounts of sulfate. Improved extraction methods were thus used to prepare two more CMA samples. These two CMA samples were prepared from whey broth containing calcium acetate as follows: the broth containing ~4% acetate was acidified to pH 3 with sulfuric acid. After removing the precipitate, the broth was extracted with the amine extractant and then back-extracted with CaO-MgO slurry. One sample (Sample #3) was obtained from continuous extraction process using the packed-column extractor described previously and the other sample (Sample #4) was prepared from batch extraction. After removing the unreacted dolime by centrifugation, the CMA-containing solution was dried and the solids were crushed to powder. These samples were then tested for acetate content and ice penetrating ability.

5.2.2 CMA Sample from Glacial Acetic Acid

For comparison purposes, a CMA sample (Sample #5) was prepared by directly reacting acetic acid with dolomitic lime (this is the present commercial production method for CMA deicer). After removing the unreacted solids, the solution was dried and the solids were crushed to powder.

5.2.3 CMA Sample from Process 2

Another CMA sample (Sample #6) was prepared from the fermentation broth without going through extraction. Sweet whey permeate was fermented in a bioreactor containing both homolactic and homoacetic bacteria to convert lactose to acetate. CaO and MgO were used to neutralize the acetic acid during the fermentation. The initial lactose concentration in the whey medium was ~3.5% and the final acetic acid concentration was ~3%. After removing cells and unreacted CaO/MgO by sedimentation, the whole broth was dried to obtain an unrefined (crude) CMA sample.

5.3 CMA SAMPLE ANALYSIS AND TESTING

5.3.1 Composition Analysis

The first two CMA samples were tested for their CMA and insoluble contents and deicing ability. Weighted samples were dissolved in water. The solution was filtered to remove any insolubles and the filtrate was analyzed with HPLC to determine acetate content. The filter paper used in filtration was washed and then dried. The dry weight difference of the filter paper before and after filtration was measured to determine the amount of insoluble in the CMA sample. Sample #1 has ~60% CMA and 30% insoluble, Sample #2 has ~75% CMA and 0% insoluble, and the commercial CMA product has ~90% CMA and 7% insoluble (Table 5.1). The large amount of insoluble in Sample #1 is believed to be CaO and MgO residues that were not reacted during back-extraction. The insoluble residues can be easily removed by filtration or centrifugation as evidenced in Sample #2, which does not have any insoluble. However, about 10% of Sample #1 and 25% of Sample #2 are believed to be $MgSO_4$. This impurity was carried into the final CMA product due to the use of sulfuric acid to acidify the acetate broth during extraction. The product purity from the extraction process was later improved by avoiding sulfate contamination in the acetate broth.

Table 5.1. Compositions of Various CMA Samples.

Content	Commercial CMA	Sample #1	Sample #2
CMA	~90% (wt/wt)	~60%	~75%
Insoluble*	~7% (wt/wt)	~30%	-
Others#	-	~10%	~25%

*mainly CaO-MgO
#include moisture and possibly $MgSO_4$.

5.3.2 Deicing Test

The ice penetration test described in SHRP (H-205.3) was carried out to test the deicing ability of various CMA samples. The experiment was done at 0°C and -15°C. Salt and dolime were also tested for comparison purposes. A water-soluble blue dye and a deicer (in powder form) were spread to cover the surface of the ice formed in a well. The same weight of each deicer sample was used in this experiment. The color penetrating depth as a function of time after applying the deicer was recorded. Each sample test was duplicated and the average was plotted and shown in Figure 5.2. It is clearly shown that salt is the fastest deicer and dolime does not work as a deicer. Sample #2 and commercial CMA have about the same deicing performance, while Sample #1 is slightly inferior due to the large insoluble content in it.

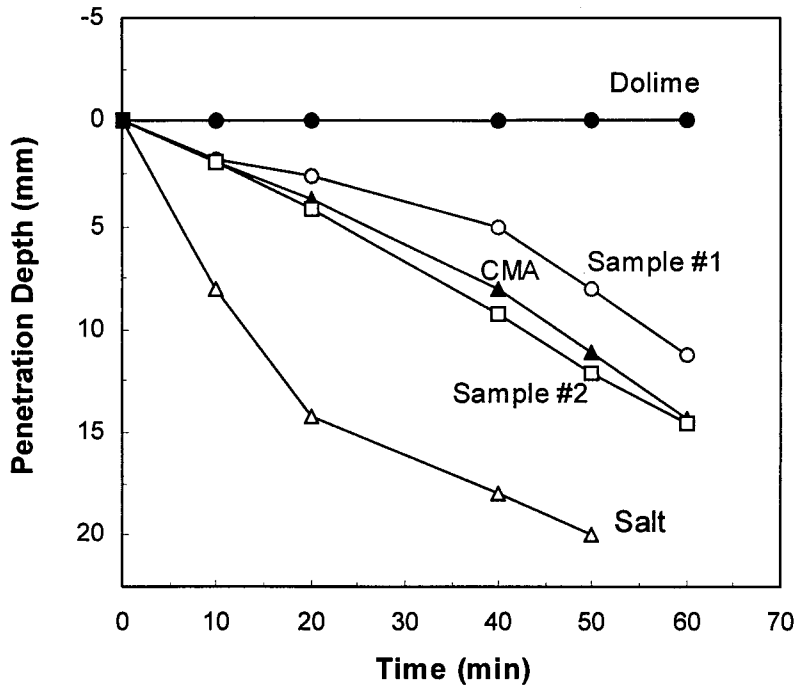


Figure 5.2. Ice Penetration Performance of Various Deicers at 0°C.

Table 5.2. Comparison of Acetate Content and Ice Penetrating Rate of Various CMA Samples.

Sample	Composition (w/w %)			Relative Ice Penetrating Rate
	Acetate	Ca	Mg	
Commercial CMA deicer	67.2	8.38	10.62	1.0
Sample #1- synthetic medium	44.8	-	-	0.81 ± 0.11
Sample #2 - acid whey	56.0	-	-	1.04 ± 0.02
Sample #3 - continuous extraction	64.8	14.41	2.62	0.99 ± 0.01
Sample #4 - batch extraction	70.1	21.34	0.095	0.88 ± 0.02
Sample #5 - direct reaction	70.2	14.55	7.04	0.97 ± 0.03
Sample #6 - whole fermentation broth	50.0	13.04	3.31	1.03 ± 0.02

*Sample #1 also contained ~30% insolubles (mainly CaO and MgO)
Sample #2 also contained ~25% $MgSO_4$

5.3.3 Comparison to Commercial CMA

The other four CMA samples were also tested for their acetate content and deicing ability. The ice penetrating rate for each deicer was estimated from the ice penetration data (not shown), and the relative rate (with commercial CMA = 1) is reported in Table 5.1. Sample #3 has about the same acetate content as the commercial CMA deicer. The slightly lower acetic acid content per gram of the solid sample can be attributed to the higher calcium/magnesium ratio in this sample. The commercial CMA has 3:7 ratio in Ca:Mg. The ice penetration experiment showed that this sample is at least as good as the commercial CMA deicer. Sample #4 is slightly inferior in its deicing ability. It is not clear why this sample is not as good as the other sample. There might have been some procedural errors in preparing this sample using batch extraction. Sample #6 is slightly better in its ice penetrating performance, probably due to the presence of other small molecular weight salts. Its acetate content is only ~70% of the pure CMA sample. This is not unexpected since lactose accounts for ~70% of the total solids in whey. In summary, CMA samples from both processes showed a similar ice penetration rate to that of the commercial CMA. The CMA made from cheese whey should perform as well as the current CMA deicer made from glacial acetic acid.

5.3.4 Conclusion and Summary

CMA samples produced from fermentation and extraction have been tested for their deicing property and acetate content. Two CMA samples, one from extraction and one from whole fermentation broth, gave ice penetration rates similar to or slightly better than that of a commercial CMA product.

VI. COST, MATERIALS BALANCE, AND MARKET ANALYSIS

6.1 INTRODUCTION

An early study conducted by Stanford Research Institute estimated that the CMA production costs from corn using *C. thermoaceticum* to ferment glucose to acetate were $0.266/lb ($0.585/kg) for the 100 tons/day (90.9 tonnes/day) plant and $0.188/lb ($0.413/kg) for the 1000 tons/day (909 tonnes/day) plant (Marynowski et al., 1985). Depending on the plant size, the raw material costs associated with the feedstock used in the fermentations accounted for 30-50% of the total production costs. Intuitively, any costs associated with the raw materials will be transferred to the product cost on a one-to-one basis. It is thus compelling to use waste materials to produce CMA (Dynatech, 1988). Also, the CMA production costs were found to be more sensitive to the operating costs than to the capital costs. Increasing the acetate concentration from fermentation is especially important for reducing the operating costs when energy-intensive evaporation and distillation are used in product recovery (Busche et al., 1982). Similar conclusions were also reached from a more recent study (Wiegel et al., 1991).

Another recent study using either sewage sludge or woody biomass to produce CMA showed the production costs would be $0.117/lb ($0.257/kg) if the residue biomass costs $50/ton ($55/tonne), or $0.092/lb ($0.202/kg) if the biomass is free (Trantolo et al., 1990). Anaerobic digestion with 6.5-day fermentation time to reach 3% acetate were assumed in this process evaluation. The product yield from fermentation was assumed to be 50%, and extraction with trioctylphosphine oxide (TOPO) was used for acetate recovery and reaction with lime (CaO) to make calcium acetate. The plant size in this analysis was 500 tons/day (454 tonnes/day) CMA.

Based on the cost relationships and financial analyses provided from these previous studies, the CMA production costs could be easily reduced to $0.09-0.15/lb ($0.20-0.33/kg) or less by using zero-cost raw material such as whey permeate. Since the fermentation results from this study are either as good as or better than all of these previous studies, it is conceivable that a low-cost CMA deicer can be produced from whey permeate using the proposed fermentation process. In this study, we investigated two CMA processes shown in Figure 5.1 and one potassium acetate process that potentially can be scaled up for commercial uses. Cost analysis for CMA production from whey permeate was based on a plant scale of processing 1.0 million lb whey permeate per day, which is equivalent to a CMA production capacity of about 24 tons/day or 8600 tons/yr. The potassium acetate process was studied for a production scale of 7800 gal/day or 2.8 MM gal/yr (50% liquid potassium acetate). This is equivalent to daily processing of 0.5 million lb whey permeate. These plant scales are chosen based on the present and immediate future market sizes for these deicers and available cheese whey supplies from typical dairy plants. A large cheese manufacturing plant typically generates 0.5 to 2 million lb whey per day.

6.2 MATERIALS AND ENERGY BALANCE

6.2.1 Materials Balance

The raw materials required to produce 24.2 tons CMA (5% moisture) and their costs are listed in Table 6.1. For KA production, the raw materials and their costs are listed in Table 6.2.

Table 6.1. Materials/Costs for Production of 24.2 Tons CMA from 1MM lb Whey Permeate.

Materials		Process #2		Process #1	
Raw materials	Price	Quantity	Costs	Quantity	Costs
Whey lactose*	2.85¢/lb	45,000 lbs	$1280	45,000 lb	$1280
CSL, 45% T.S.	$45/ton	10 tons	$ 450	10 tons	$ 450
Lime, CaO	$40/ton	-	-	8 tons	$ 320
H_2SO_4	$30/ton	-	-	15.2 tons	$ 460
Dolomitic lime	$20/ton	7.5 tons	$ 150	7.5 tons	$ 150
Solvent	$ 2/lb	-	-	25 lb	$ 50
Waste material					
$CaSO_4$	$25/ton	-	-	20.6 tons	$ 515
Total material costs		$1880 or $78/ton		$3225 or $133/ton	

Table 6.2. Materials and Their Costs for the Production of 7300 Gallons Potassium Acetate (50%) from 0.5 MM lb Whey Permeate.

Materials		Quantity	Costs
Raw materials	Price		
Whey lactose*	2.85¢/lb	22,500 lb	$ 640
Casein hydrolysate	$1.0/lb	750 lb	$ 750
Lime, CaO	$40/ton	4 tons	$ 160
H_2SO_4	$30/ton	7.6 tons	$ 230
KOH	$0.4/lb	19240 lb	$7700
Solvent	$2.0/lb	25 lb	$ 50
Waste material $CaSO_4$	$25/ton	10 tons	$ 250
Total Material Costs		$9780 or $1.34/gal	

*Based on 2 cents/lb total solid (70% lactose) for whey permeate.
2.2 lb = 1 kg; 2200 lb = 1 tonne; 1.1 tons = 1 tonne.

Depending on plant situations, the whey permeate (1 MM lb liquid, 6.5% total solid, 4.5% lactose) cost ranges from -2 to -4 cents per pound T.S. (for plants to dispose) to 2 cents pr pound T.S. (for plants to receive). Concentrated corn steep liquor (CSL, 45% T.S.) is added at ~2.2% to whey permeate for fermentation (experiments showed from 1.5% to 2.5% is appropriate). This CSL can be substituted with casein hydrolysate (at ~0.15%), if desired (such as for potassium acetate production). Solvent loss is estimated at 25 lb, based on its water solubility (5 ppm) times a factor of 5. Depending on locations, dolomitic lime or limestone may be available at no cost.

The solid waste, $CaSO_4$, generated from Process #1 can be disposed by landfilling at an estimated cost of $25/ton. The treatment cost for the liquid waste stream from Process #1 should be insignificant since its BOD is low. However, the solid waste can be eliminated if an extractive fermentation is implemented.

6.2.2 Energy Balance

Table 6.3 lists all energy and utilities used in the CMA production processes. The energy input for fermentation is minimal, since there is no aeration or agitation used in the anaerobic fermentation (at 37°C). Steam heat is used mainly in evaporation to remove water. It is assumed that one lb_m steam can remove 3 lb_m (6.6 kg) water for triple-effect evaporators. Only small amounts of steam will be needed in equipment and medium sterilization. Non-sterile or pasturized whey permeate will be acceptable for fermentation. Natural gas is used to generate hot air in drying. It is assumed that to remove one lb water (the heat of evaporation=1050×10^{-3} megajoules/lb) would require one ft^3 natural gas (1330 Btu/ft^3, with 75% efficiency). Electricity is used in pumping and other operations. Water is not considered since condensed water from evaporation and drying can be recovered and used for various processing purposes.

The energy requirements for the potassium acetate process are small. Since there is no evaporation or drying operation involved in this process, the cost for electricity is the only major energy cost. This is estimated at 1500 kWh or $75 per day for the plant size studied.

Table 6.3. Energy/Utility Costs for Production of 24.2 Tons CMA from 1 MM lb Whey Permeate.

Energy		Process #2		Process #1	
Utilities	Price	Quantity	Costs	Quantity	Costs
Steam	$3/1000 lb_m	280,000 lb	$ 840	-	-
Natural gas	$5/1000 ft^3	94,000 ft^3	$ 470	152,000 ft^3	$ 760
Electricity	$0.05/kWh	2000 kWh	$ 100	3000 kWh	$ 150
		Total	$1410	Total	$ 910
Total Energy Costs		$58.3/ton		$37.6/ton	

2.2 lb = 1 kg; 2200 lb = 1 tonne; 35.7 cu ft = 1 cubic meter; 0.28 kwh = 1 megajoule.

6.3 ECONOMIC AND COST ANALYSES

Unless otherwise noted, all cost analyses discussed here are for a plant processing 1,000,000 lb whey permeate (4.5% lactose) to produce ~24 tons CMA (5% moisture) per day.

6.3.1 Equipment Costs and Capital Investment

The purchasing costs for major equipment used in the two CMA production processes discussed previously are listed in Table 6.4. They were estimated either from vendor quotations or using the

data presented in the book, Peters and Timmerhaus (1991) "Plant Design and Economics for Chemical Engineers" 4th edition, McGraw-Hill, New York. The detailed discussion of each unit operation step and associated equipment design in the production process can be found in the Phase I Interim Report.

As shown in Table 6.4, for both processes the major equipment purchasing costs would be the bioreactor (~42% of the total costs). The next high-price items are the dryer (~20%), the evaporator (~29%, for Process 2), and extractors (24%, for Process 1). However, the dryer and evaporator may be available from an existing whey processing plant.

Other direct costs for equipment installation, instrumentation and control equipment, service facilities, buildings, and land for the plant are estimated based on percentages of the total major equipment purchasing costs shown in Table 6.4. The percentages used in this analysis are also listed in Table 6.5.

Table 6.4. Costs for Major Equipment Items.

Equipment (number; size)	Process 2	Process 1
Whey holding tank (250,000 gal)	$ 75,000	$ 75,000
Bioreactor (5; 30,000 gal)	$750,000	$750,000
Evaporator (triple-effect)	$498,000	-
Mixing tank for acidification (2,500 gal)	-	$ 20,000
Extraction column	-	$350,000
Mixing tank for back-extraction (5,800 gal)	-	$ 22,000
Settling (separation) tank (25,000 gal)	-	$ 52,000
Dryer (spray)	$330,000	$400,000
Granulizer (uncertain)	$ 22,000	$ 22,000
Pumps (centrifugal, 6 or more)	$ 60,000	$ 86,000
Subtotal	$1,735,000	$1,777,000

Table 6.5. Cost Estimation as Percentage of Purchased Equipment.

Other costs as percentage of total major equipment purchasing costs	
Installation	35%
Instrumentation & control equipment	10%
Service facilities	15%
Piping	10%
Electrical	5%
Buildings	20%
Land	5%
Total	**100%**

The indirect costs (including engineering and supervision, construction expense, contractor's fee, and contingency) are estimated at 20% of the total direct costs. The total fixed-capital investment is thus 240% of the total major equipment purchasing costs, or $4,164,000 for Process #2 and $4,264,800 for Process #1. If the working capital is at 15% of the fixed-capital investment, the total capital investment will be $4.8 million for Process #2 and $4.9 million for Process #1.

For liquid potassium acetate, no dryer or pelletizer is needed. At half of the CMA plant scale, the total capital investment for the plant would be approximately $2.4 million.

6.3.2 Production Costs

The estimated daily production costs for processing 1.0 million lb whey permeate (containing 4.5% lactose) to 24.2 tons CMA deicer (containing 5% moisture) are listed in Table 6.6. As expected, Process #2 is cheaper than Process #1. The product cost for Process #2 is $290.9/ton ($320/tonne) and $328.5/ton ($361/tonne) for Process #1. These costs are significantly lower than the present market price of ~$1000/ton (~$1100/tonne), of which 75% is attributed to the cost of glacial acetic acid (present spot market price: ~$0.79/lb or $792/tonne).

Table 6.6. Estimated Daily Operating Costs for Plants to Produce 24.2 Tons CMA Deicer from 1.0 MM lb Whey Permeate Per Day.

	Process 2	Process 1
I. Manufacturing cost		
A. Direct production costs	$ *4096*	$ *4947*
1. Raw materials	$ 1880	$ 2710
2. Energy & utilities	$ 1410	$ 910
3. Waste disposal	-	$ 515
4. Labor	$ 500	$ 500
5. Maintenance & repairs (2% of FCI per yr)	$ 228	$ 234
6. Operating supplies and laboratory charges	$ 63	$ 63
7. Patents and royalties	$ 15	$ 15
B. Fixed charges	$ *1483*	$ *1519*
1. Depreciation (10 yr linear or 10% of FCI per yr	$ 1141	$ 1168
2. Local taxes (2% of FCI per yr)	$ 228	$ 234
3. Insurance (1% of FCI per yr)	$ 114	$ 117
C. Plant overhead costs (50% of A.4 & A.5)	$ *364*	$ *367*
II. General expenses		
A. Administration costs (15% of I.A.4 & I.A.5)	$ *109*	$ *110*
B. Distribution and selling costs	$ *200*	$ *200*
C. Financing (interest) (6% of total capital investment)	$ *787*	$ *806*
Total product cost (I + II)	**$ 7039**	**$ 7949**
Product cost per ton	$ 290.9	$ 328.5

1. Process 2 is via evaporation shown Fig. 5.1 and Process 1 is via extraction shown in Fig. 5.1.
2. FCI: fixed-capital investment.

However, the CMA product from Process #2 also contains large amounts of other materials (~30% in weight), which also contribute to deicing performance (see Sample Testing section). Thus, on the total solid basis, this product cost is ~$203.6/ton ($224/tonne). For these CMA processes, the total direct production costs consist of ~60% of the product cost. About 49.3% (Process #2) and 44.5% (Process #1) of product cost are sensitive to the plant size. Thus, the unit product cost will change with the process scale.

The estimated production costs for producing liquid potassium acetate deicer are shown in Table 6.7. The analysis is based on 0.5 million lb whey permeate or 7300 gallons (27630 liters), 50% (w/v) liquid potassium acetate per day. The major production cost in this process is the raw material cost for potassium hydroxide, about 80% of the product cost. The product cost is estimated at $1.66/gal ($0.44/liter), which is much lower than the present market price of ~$4.35/gal ($1.15/liter). The economics for this process are relatively insensitive to process scale since only 16.4% of the product cost is scale-dependent.

Table 6.7. Estimated Daily Operating Costs for a Plant to Produce 7300 Gallons 50% (w/v) Liquid Potassium Acetate Deicer from 0.5 MM lb Whey Permeate Per Day.

I. Manufacturing cost	
A. Direct production costs	*$10447*
1. Raw materials	$ 9530
2. Energy & utilities	$ 75
3. Waste disposal	$ 250
4. Labor	$ 400
5. Maintenance & repairs (2% of FCI per yr)	$ 114
6. Operating supplies and laboratory charges	$ 63
7. Patents and royalties	$ 15
B. Fixed charges	*$ 742*
1. Depreciation (10 yr linear or 10% of FCI per yr	$ 571
2. Local taxes (2% of FCI per yr)	$ 114
3. Insurance (1% of FCI per yr)	$ 57
C. Plant overhead costs (50% of A.4 & A.5)	*$ 257*
II. General expenses	
A. Administration costs (15% of I.A.4 & I.A.5)	*$ 78*
B. Distribution and selling costs	*$ 200*
C. Financing (interest) (6% of total capital investment)	*$ 394*
Total product cost (I + II)	**$12118**
Product cost per gallon	$ 1.66

6.3.3 Return of Investment

Table 6.8 shows the rate of return of investment (R.O.I.) for Process 1 and Process 2 for various CMA product selling prices. The analysis was based on 25% tax rate on profit. Apparently, these processes will be economical at a CMA price of $350/ton ($385/tonne) or higher. For a minimum of 20% return rate, the CMA prices would be $450/ton ($495/tonne) to $500/ton ($550/tonne). However, it may be more profitable to produce liquid potassium acetate deicer. Even at a sale price of $2.5/gal ($0.66/liter), the R.O.I. is still at ~70%.

Table 6.8. Economics of CMA Production from Whey Permeate.

	Process 2	Process 1
Product Cost	$ 290.9/ton	$328.5/ton
Total Capital Investment	$ 4.8 MM	$ 4.9 MM
Rate of R.O.I.		
@ $600/ton CMA	42.7%	36.7%
@ $500/ton CMA	28.9%	23.2%
@ $400/ton CMA	15.1%	9.7%
@ $350/ton CMA	8.2%	3.0%

1.1 tons = 1 tonne.

6.3.4 Effect of Process Scale

The effects of process scale on the product cost and economics are analyzed based on the following three assumptions:

1. Costs related to equipment or fixed-capital investment are related to the process scale by a cost (exponential) factor of n = 0.6.
2. Labor costs are related to the process scale by a cost factor of n = 0.5.
3. All other direct production costs and distribution and selling costs remain unchanged on the per ton CMA basis.

The effects of process scale on product cost are illustrated in Table 6.9. It is clear that the potassium acetate product cost is not sensitive to process scale, while CMA product costs would increase dramatically if the process scale is less than 25% of the one studied before. Thus, the minimum process scale for CMA production from whey is 6 tons/day (2200 tons/yr) or 250,000 lb whey/day (113.6 tonnes whey/day).

6.3.5 Liquid CMA

It is also noted that for both processes, energy costs and capital costs for drying are high. If 30% liquid CMA is to be produced as the final product (used as liquid anti-icing agent), the CMA

product cost can be reduced by $50/ton ($55/tonne) (dry wt) CMA, or ~$241/ton ($265/tonne) for CMA present in the fermentation broth (Process #2 without drying) and ~$279/ton ($307/tonne) for CMA solution (Process #1 without drying).

Table 6.9. Effect of Process Scale on CMA Product Cost.

Relative Scale	CMA Process 2 (Cost/ton)	CMA Process 1 (Cost/ton)	K-acetate (Cost/gal)
2.0	$256.2	$293.1	$ 1.59
1.5	$269.4	$306.6	$ 1.62
1.0	$290.9	$328.5	$ 1.66
0.75	$304.8	$346.3	$ 1.69
0.5	$336.7	$375.2	$ 1.78
0.25	$397.2	$436.8	$ 1.86
0.1	$507.7	$549.5	$ 2.07

1.1 tons = 1 tonne. 0.264 gal = 1 liter.

6.3.6 Extractive Fermentation Process

In Process #1, CaO and sulfuric acid are used to control the broth pH during fermentation and extraction. The costs associated with these raw materials plus the disposal cost for the waste $CaSO_4$ generated from them account for $53.5/ton ($58.9/tonne) CMA produced. This cost may be eliminated if an extractive fermentation process can be successfully developed. Potentially, the CMA product cost could be lowered to $223/ton ($245.3/tonne) for solid CMA and $173/ton ($190.3/tonne) for liquid CMA (30% solution) by using extractive fermentation. Variations of CMA product cost as affected by various aforementioned factors are summarized in Table 6.10.

Table 6.10. Variations in CMA Product Cost.

Product	Process #2 (Crude CMA)	Process #1 (Refined CMA)	Extractive fermentation
Solid CMA (5% moisture)			
whey lactose @$0.028/lb	$291/ton	$329/ton	$276/ton
whey lactose @$0.000/lb	$238/ton	$276/ton	$223/ton
Liquid CMA (30% soln)			
whey lactose @$0.028/lb	$241/ton	$279/ton	$226/ton
whey lactose @$0.000/lb	$188/ton	$226/ton	$173/ton

2.2 lb = 1 kg; 1.1 tons = 1 tonne.

6.3.7 Effect of Capital Costs

The capital costs may vary greatly depending on equipment selected and available support facilities. The capital-related product cost consists of approximately 37.1% for CMA Process 2, 33.7% for Process 1, and 10.8% for the potassium acetate process. The impacts of increasing or decreasing capital costs (equipment purchasing, installation, etc.) on the product cost are illustrated in Table 6.11. It is clear that even if the capital costs are doubled for the same production scale studied before, the CMA product costs are still much lower than the current market price.

Table 6.11. Effects of Capital Costs on CMA Product Costs.

Cost factor	Process 2 ($/ton)	Process 1 ($/ton)	K-acetate ($/gal)
0.5	236.9	273.2	1.57
0.8	269.3	306.4	1.62
1.0	290.9	328.5	1.66
1.2	312.5	350.6	1.70
1.5	344.9	383.8	1.75
2.0	398.8	439.1	1.84

$1/ton = $1.1/tonne; $1/gal = $0.26/liter.

6.4 MARKET ANALYSIS

6.4.1 Comparison of CMA to Other Deicers

Although CMA has been proven to be as effective as rock salt in road deicing and as an effective anti-icing agent, it is currently only used in limited areas due to its high price. A lower-cost CMA deicer (from cheese whey) should allow CMA to compete better with other chemical deicers (NaCl, $CaCl_2$, Cargill CG-90, and urea) and increase CMA use in deicing. It is noted that the use of or demand for chemical deicers is very sensitive to the winter weather and may vary as much as by a factor of two or three from year to year.

All solid chemical deicers, except for salt, cost ~$150/ton ($136/tonne) or higher, but CMA at $1000/ton ($1100/tonne) is the most expensive one at the present time. However, all of these lower-cost deicers are more corrosive than CMA. Also, CMA is a corrosion inhibitor. The 20% CMA/80% salt mixture in solution has been shown to be almost as non-corrosive as the CMA solution. Thus, it is possible to make a deicer consisting of 20% CMA and 80% salt with a competitive price of ~$150/ton ($165/tonne). In addition, the federal fund will pay states for 80% of the CMA cost used in deicing new bridges and highways in environment-sensitive areas. The costs for CMA users in these applications thus will be only 20% of the purchasing price. When the low-cost CMA deicer from cheese whey, at a projected price of $400/ton ($440/tonne) or lower, is available, the CMA deicer will be much cheaper to use than most of the other low-corrosion deicers. However, so far the federal cost sharing alone has not increased CMA use.

6.4.2 CMA Market Survey

6.4.2.1 Current Use of CMA in Highway Deicing

In order to determine CMA market size and price effects on its market acceptance, a CMA market survey to 10 state transportation departments was conducted in 1993. Only state transportation departments were surveyed because they are the major (potential) users of CMA and most of current CMA use is for highway deicing. Of the 10 state transportation departments surveyed, eight have used CMA deicer in the past, but only three used CMA in the '92/'93 winter season. Five states were planning to use CMA again in the 1994 winter season. The amount of CMA used (or to be used) by each state ranges from 50 tons (45.4 tonnes) to 200-400 tons (181.8-363.6 tonnes), depending on the weather conditions. The reasons for these state transportation departments to use CMA in the past included: 1. to prevent corrosion (6 responses), 2. to prevent environmental damage (2 responses), 3. to support SHRP research (1 response), 4. laws banning the use of salt on new bridges (1 response). The price of CMA is the most important factor affecting their future use of CMA. The next important factor is the deicing performance (6 responses), followed by corrosion (2 responses) and laws (1 response). Environmental damage by salt is not considered as important in their future decision on using CMA deicer.

6.4.2.2 Factors Affecting CMA Use

According to a 1991 Transportation Research Board report, the costs of salt use associated with infrastructure and automobile damage were ~$555/ton ($610/tonne). However, this information did not seem to affect CMA use in highway deicing (4 No, 6 Maybe), especially at the CMA price of $650/ton ($715/tonne) in 1993. Again, all respondents did not seem to pay much attention to the environmental benefit from CMA. The 1991 ISTEA providing states with 80% of CMA costs did not have any significant impact on CMA use, either (8 No, 1 Yes, 1 Maybe), because federal funds are needed for highway construction and rehabilitation. Also, to use 20% CMA and 80% salt mixture as a deicer to reduce both costs and corrosion did not seem increase CMA use (6 Maybe, 3 No, 1 was the user before). Only 50% of the respondents were interested in anti-icing using CMA (5 Yes, 5 No). However, the anti-icing application is likely to increase CMA use by 63%, based on the estimated CMA uses in anti-icing and in deicing.

The low-level interest in these potentially cost-effective applications might be attributed to 1) lack of education on cost benefits of CMA deicer, and 2) no driving force for change. The seemingly high CMA price again was the major obstacle for CMA use in highway deicing. The price resistance may be much lower in the consumer product market if the CMA/salt blend works as effectively as salt. There are laws or public demands banning salt use on new bridges and in environmentally sensitive areas that have helped CMA use, but the effect is relatively small. It is clear that a much lower priced CMA is the only hope for increasing CMA use in highway deicing at the present time and in the foreseeable future.

6.4.2.3 Effect of CMA Price on Market Size

Eight respondents provided their estimated CMA use at various price levels. Only one respondent indicated that price would have a slight effect for the range between $400 and $650. The price for

CMA to start to break in highway deicing market is most likely at $300/ton ($330/tonne) (6 responses) or lower ($220/tonne, 1 response; $110/tonne, 1 response). Two respondents indicated that at the break-in price, the amount of CMA used could increase from zero or 200 tons (181.8 tonnes) to 50,000-100,000 tons (45,454-90,909 tonnes). It is thus clear that the price effect on CMA use will not be significant until it is at $300/ton ($330/tonne) or lower. Based on the responses from this survey, CMA market size as affected by its price was estimated (Fig. 6.1).

Figure 6.1 illustrates the effect of CMA price on CMA market size normalized on either usage (tonnage) or market value ($MM) with the $650/ton ($715/tonne) price in 1993 being at 1. The present CMA price is $1000/ton or $1100/tonne. Figure 6.1a gives a more conservative view while Figure 6.1b provides a more aggressive view (based on a quantum jump in CMA use at break-in price). It is clear that there is no commercial benefit to lower CMA price to $400/ton ($440/tonne), especially in the highway deicing market, at the present time. This market survey did not cover other (potential) uses for CMA, such as in private property, airport, and municipality deicing, which may have a greater acceptance for a higher priced CMA. These markets may provide better opportunities for small, local CMA producers.

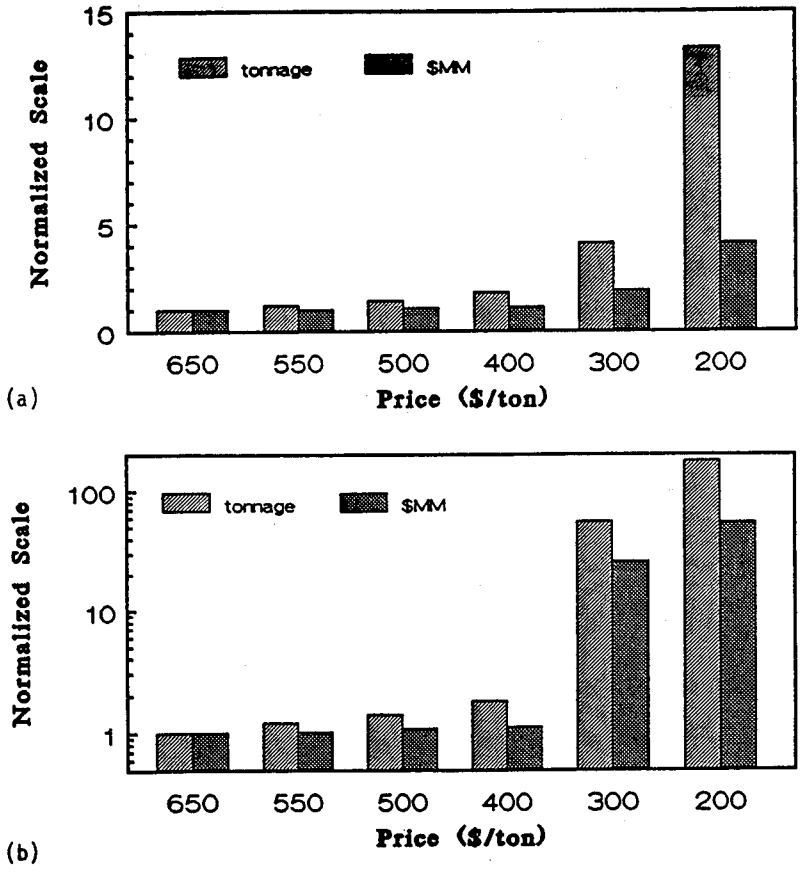


Figure 6.1. Effect of CMA Price on Projected Market Size: (a) Conservative View; (b) Aggressive View.

6.4.3 Market and Supply of Whey

Each year in the United States, about 28 billion lb of liquid whey are not utilized or even properly treated due to the lack of an economically viable technology. The proposed process potentially can produce ~1.7 billion lb (or 0.77 million tons) of low-cost CMA deicers from the currently unused whey. However, not all the unused whey is available at an economical scale. There are numerous cheese plants generating less than 150,000 lb (68.18 tonnes) liquid whey per day, but only a few plants with a capacity of 1 MM lb (454.5 tonnes) whey or more. The typical size plants generate 200,000 lb (90.9 tonnes) to 500,000 lb (227.3 tonnes) whey per day. The large plants usually process their whey to whey protein concentrate, lactose, and/or whey powder (see Figure 2.1), and have little or no wastes. However, the lactose operation is not profitable at low market prices (<$0.33/kg). The medium-size plants are feeding their whey to animals, if possible, at a break-even cost. But the animal feed market is not always there. The concentrated whey delivered to the users is priced at 1-3 cents/lb whey solid. However, the costs for concentrating and transporting whey to animal feeders at distance are high, amounting to a loss of 2-4 ¢/lb (4.4-8.8 ¢/kg) whey solid.

There are cheese plants that are located close together (within 100 miles) and they together will provide an economic scale for CMA production. About 20 cheese plants (in New York, Vermont, Ohio, and Wisconsin) were surveyed about their annual production of whey and its current uses and disposal means. The response rate to this survey was 50%. In general, the results are as expected. Most plants responding to the survey indicated their immediate need for better utilization of whey byproducts. All are interested in producing CMA deicer from whey (or equivalent waste streams), if the process is economical and available for commercialization. Depending on the plant size, the available whey from each plant ranges from less than 1 million lb/yr to more than 300 million lb/yr (136,360 tonnes/yr). Some typical plant situations are summarized in Table 6.12.

Table 6.12. Current Situations for Some Dairy Plants Producing Whey.

Plant	Amt. of whey	Current uses or disposal
Kraft Gen. Foods Lowville, N.Y.	10 MM lb solid or 150 MM lb liq./yr	Feed operation; landspreading
McCadam Cheese Chateaugay, N.Y.	221 MM lb liq./yr	Whey protein concentrate Landspreading 188 MM lb whey permeate
Friendship Dairies Friendship, N.Y.	60 MM lb liq./yr	4 MM lb dry whey powder landspreading
Avonmore Cheese Monroe, Wisconsin	30 MM lb solid or 450 MM lb liq./yr	Whey protein concentrate; landspreading Whey permeate - 12.5 MM lb solid
Brewster Dairy Brewster, Ohio	315 MM lb liq./yr	Whey protein concentrate; lactose; 380 MM lb whey permeate 13 MM lb de-lactose permeate (28% lactose)

1 MM lb = 454.5 tonnes.

The cheese plants in Vermont that responded to this survey are relatively small. About 75% of the whey produced in Vermont (42 MM lb solid/yr or 19,090 tonnes solid/yr) is shipped to Vermont Whey Company (Wyeth Nutritionals, Inc.), which pays 2 ¢/lb (4.4 ¢/kg) solid (~70% lactose) for the whey it receives. All of this whey is presently processed to whey powder, whey protein concentrate (WPC), lactose, or reduced mineral whey for human consumption, with little waste left over. However, the human food market for whey is saturated in the United States.

It is thus clear that whey lactose is available at zero to ~2.8 ¢/lb (5.5 ¢/kg) for CMA or acetate production. For a plant processing up to 0.5 MM lb (227.3 tonnes) whey per day, whey lactose could be supplied free from a single cheese plant. For 1 MM lb (454.6 tonnes) and larger whey plants, the cost of lactose can be estimated at 2.8 ¢/lb (5.5 ¢/kg).

6.4.3.1 Other New Uses of Whey

There are other new uses being researched for whey and whey permeate. Although some of them (such as lactic acid and calcium propionate) have been or are close to being commercialized, none of these new uses are likely to consume all the present surplus whey. The optimal choice for product selection will be dependent on the plant size, available technologies, and market situations. Apparently, there will be no universal solution for the whey disposal (utilization) problem; CMA, however, seems to be an attractive choice. Furthermore, one single plant (24.2 tons CMA, 1 MM lb whey permeate per day) would be able to produce 8,800 tons CMA per year, which is enough for, if not more than, the current market demand. Similarly, one single potassium acetate plant (27630 liters or 0.5 MM lb whey permeate per day) would satisfy 50% of the airport deicing market in the North America. Thus, supplies of whey for CMA and acetate production should be more than adequate in the foreseeable future.

6.4.3.2 Supplies of Other Raw Materials

Dolomitic lime and limestone are plentiful throughout North America, including in Ohio and New York. The number and capacity of corn wet milling plants in the United States are increasing due to increased production of ethanol, lactic acid, citric acid, xanthan gum, amino acid, and other specialty chemicals from corn dextrose. The feed market for corn wet milling byproducts, including corn steep liquor, is already saturated. Thus, large amounts of CSL will continue to be available at affordable prices, if not lower than today's cost of $40/ton-$45/ton ($44/tonne-$49.5/tonne).

6.4.3.3 Other Feedstocks for CMA Production

If 10% of the current rock salt is replaced with CMA, the projected annual use of CMA deicer would be 1-1.4 MM tons (0.9-1.3 MM tonnes). However, only ~0.77 MM tons (0.7 MM tonnes) CMA can be produced from the currently unused whey. If the CMA market should grow to more than 0.8 MM tons/yr (0.73 MM tonnes/yr), more CMA deicers can be produced from other feedstocks using essentially the same fermentation process. The homolactic bacteria used in lactose fermentation also can ferment glucose (or corn dextrose) and other fermentable carbohydrates to lactate. Thus, the same fermentation also can be used with other renewable resources, including other food processing wastes and corn-based feedstocks. However, the corn-based CMA deicer

may cost ~$100/ton ($110/tonne) more than the whey-derived CMA due to its higher raw material cost (~$0.20/kg for dextrose).

Other potential abundant feedstocks for CMA production include sewage sludge, woody (ligino-cellulosic) biomass, and waste flue gas (H_2 and CO). However, the use of these feedstocks for fermentation is not economical due to the high costs in feed pretreatments, low product yields, and low reaction rates. Their economical use as a feedstock requires economy of scale (e.g., 100,000 ton/yr or 90,909 tonne/yr) that is far beyond the present and foreseeable CMA market demands. There are also other concerns in using these waste materials, including heavy metal and chemical contamination of the feedstock and product, and heavy nutrient supplementation requirement for the fermentation.

It is thus concluded that cheese whey is the most economical feedstock for CMA and acetate deicers production at the present time and in the forseeable future.

6.5 CONCLUSION AND RECOMMENDATIONS

Low-cost CMA deicers can be produced from cheese whey via anaerobic fermentation and extraction using long-chain aliphatic amines. The CMA deicers produced from the two processes have similar deicing performance to that of the present commercial CMA deicer made from glacial acetic acid. The lowered CMA costs could dramatically increase CMA use in the deicing market.

6.5.1 Selection of Plant Site

Commercialization strategy should be developed based on plant situations and needs. To minimize transportation costs, the CMA plant should be located near whey sources and CMA users. Supplies of dolomitic lime and other feedstocks (such as corn steep liquor) also should be considered. New York state and Ohio appear to be two prime candidates for the CMA and potassium acetate plants. Both states have large dairy industries and are in the snow belt. There are several large airports both within and near these two states. Ohio also has a large corn production and processing industry.

6.5.2 Multiple Products Plant

The same fermentation and extraction technologies also can be used to economically produce other organic salts, such as calcium propionate and sodium lactate from whey. Currently, some propionate is produced for food use from whey fermentation. Also, sodium lactate is used in foods and beverages. Since at the present time the CMA market is relatively small and seasonal, it would be advisable to construct a fermentation plant that would have capabilities and flexibility to produce various products (acetate, lactate, and propionate) according to market demands. This will not only increase the plant's profitability, but also reduce investment risk when the product demand fluctuates with season and weather. For such a plant, the recycle batch process (see previous sections), which uses smaller immobilized cell bioreactors along with larger conventional agitated tank reactors, is recommended. The total capital investment for a multiple product plant would be about the same as the CMA plant since all major equipment is shared in this plant. Thus, more

CMA and potassium acetate may be produced in winter when demands are high, while sodium lactate and calcium propionate are produced in the rest of the year. Figure 6.2 illustrates this multiple products fermentation plant concept.

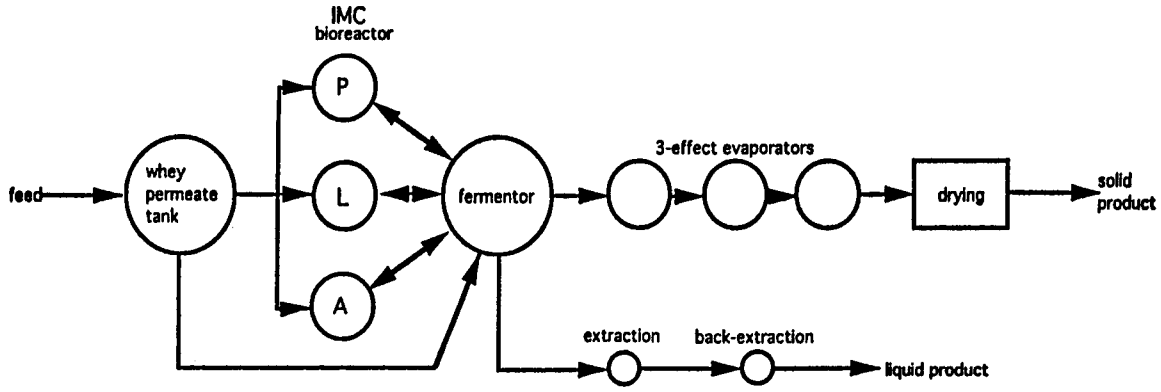


Figure 6.2. A Conceptual Flowsheet for Multiple Fermentation Products from Whey. (P – propionic acid reactor, L – lactic acid reactor, A – acetic acid reactor)

VII. CONCLUSIONS

CMA, a mixture of calcium acetate and magnesium acetate, is used as an environmentally benign roadway deicer. The present commercial CMA deicer made from glacial acetic acid and dolomitic lime or limestone is expensive compared with salt and other deicers. Also, a liquid potassium acetate deicer is used to replace urea and glycol in airport runway deicing. In this work, two alternative low-cost methods to produce these acetate deicers from cheap feedstocks, such as cheese whey and other biomass and industrial wastes, were studied.

Acetate can be produced from whey lactose with a minimal nutrient supplementation with corn steep liquor (CSL) using a co-culture immobilized in a fibrous-bed bioreactor. The acetate yield from lactose in the fermentation was 90% or higher. The final acetate concentration from the fermentation was as high as 75 g/L and the bioreactor had a productivity of 1~2 g/L·h, depending on the acetate concentration. Also, the reactor was stable for long-term operation, either as a continuous reactor or as a recycle batch or fedbatch reactor. These fermentation results indicate that commercial production of acetate from low-cost industrial feedstocks (e.g., whey permeate) and nutrient supplements (e.g., CSL) should be feasible.

A two-step extraction process involving extraction with an amine-based extractant and back-extraction with a base solution was developed for CMA production from dilute acetic acid solution. The acetic acid produced in fermentation can be effectively extracted and separated from lactic acid using a low content of a secondary amine in a long-chain alcohol. This extraction system also can be used in extractive fermentation to reduce product inhibition problems found in fermentation.

CMA deicers produced from cheese whey by fermentation and extraction were tested for their acetate content and deicing property. The CMA solid sample obtained from extraction of the acetic acid present in a dilute aqueous solution and then back-extracted with dolomitic lime to form CMA had about the same acetate content (~70% acetic acid or ~90% CMA) as the commercial CMA deicer. The sample from dried whey fermentation broth contained 50% acetic acid or ~63% CMA, with the remaining solids being other organics and salts present in whey. Deicing tests showed that CMA samples from fermentation and extraction had equal or slightly better ice penetration rates than the commercial CMA.

Cost analysis showed that CMA can be produced at a product cost of $204-$328/ton ($224-$360/tonne), less than 30% of the current market price for commercial CMA ($1,000/ton or $1,100/tonne), for a plant size of 8,400 tons CMA per year. The lower CMA cost should dramatically increase CMA use in the deicing market.

APPENDIX A. DESIGN OF PILOT-SCALE BIOREACTOR FOR FERMENTATION

A1. 150-LITER FIBROUS-BED BIOREACTOR

As the fibrous-bed bioreactor technology is very unique in terms of reactor configuration, it is not possible to obtain a commercially available fermentation system, or not economic to modify a commercially available fermentation system, to match the requirement of the fibrous- bed technology. Based on our bench top experiment and experience on the fibrous-bed bioreactor, a 150-L (total volume) stir tank bioreactor was designed (shown in Fig. A1). As schematically shown in Figure A2, two spiral-wound fibrous bed packings are installed between three marine agitators, which ensures an effective mixture within the fibrous bed. The fibrous packings were supported by Goodloe's waved stainless steel wire cloth, which ensures efficient packing performance. Furthermore, four baffles are used between the packings and the wall of the vessel to provide a vertical channel allowing an effective mass transfer between top packing and bottom packing (detailed engineering design shown in Figures A3-A7).

The bioreactor was controlled by a PC-based control system. Figures A8-A10 show schematic diagrams of the control loops for temperature and pH. With the application of computer technology (three control loops are designed that can both manually and automatically control fermentor temperature, agitation speed, and pH. The pilot scale fibrous-bed bioreactor had the following features:

- The fibrous-bed bioreactor with internal agitation (speed range from 0 to 800 rpm) improves mass transfer efficiency as compared with external re-circulation using pumps.

- With the coordination of two thermocouples applied on both top of the packing and in the middle of the packing, it is more accurate to indicate the vessel temperature at a large reactor scale, and it is also easier for temperature control. In combination with agitation and jacket temperature controller, the fibrous-bed bioreactor is able to control temperature in the range of 25 to 60°C with an accuracy of about ± 1°C (Figure A11a).

- Because of the application of an internal effective agitation system, pH control also shows a better performance to ± 0.5 (Figure A11b), and no pH gradient was observed in the bioreactor.

- The automatic control system as well as data acquisition capability allows the fibrous-bed bioreactor to be operated with minimum supervision.

The complete fermentation pilot plant also consisted of two 40-L fermentors used for inoculum and medium preparations. These fermentors were modified from refurbished NBS fermentors that had the basic functions for temperature, pH, and agitation control. A photograph of the fermentation pilot plant is shown in Figure A1. In addition, a 16-ft Karr column extractor (2-in diameter) was also equipped for production recovery (Figure A12).

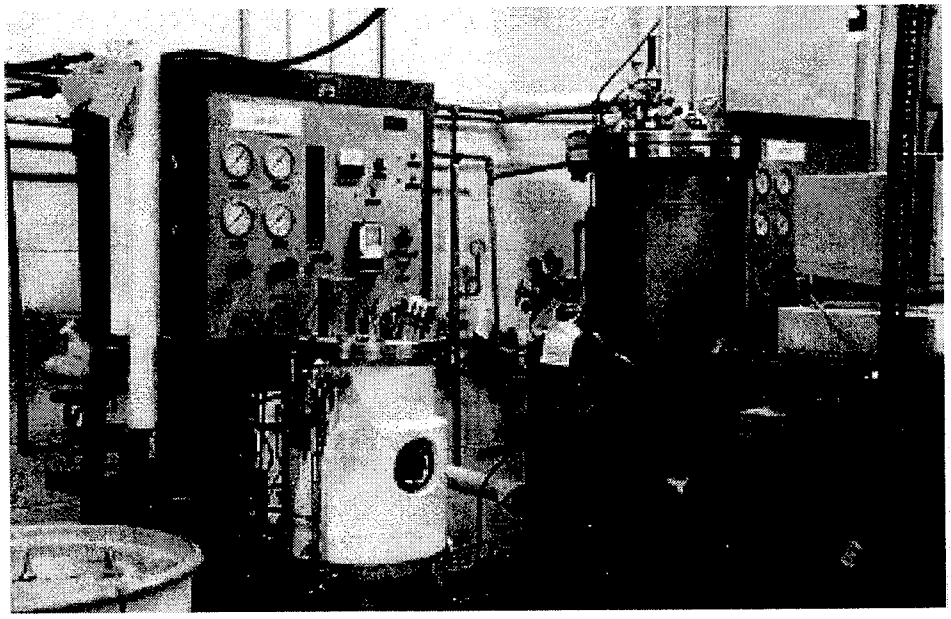


Figure A1. The 150-liter Fibrous-Bed Bioreactor and Associated Fermentation Pilot Plant Equipment.

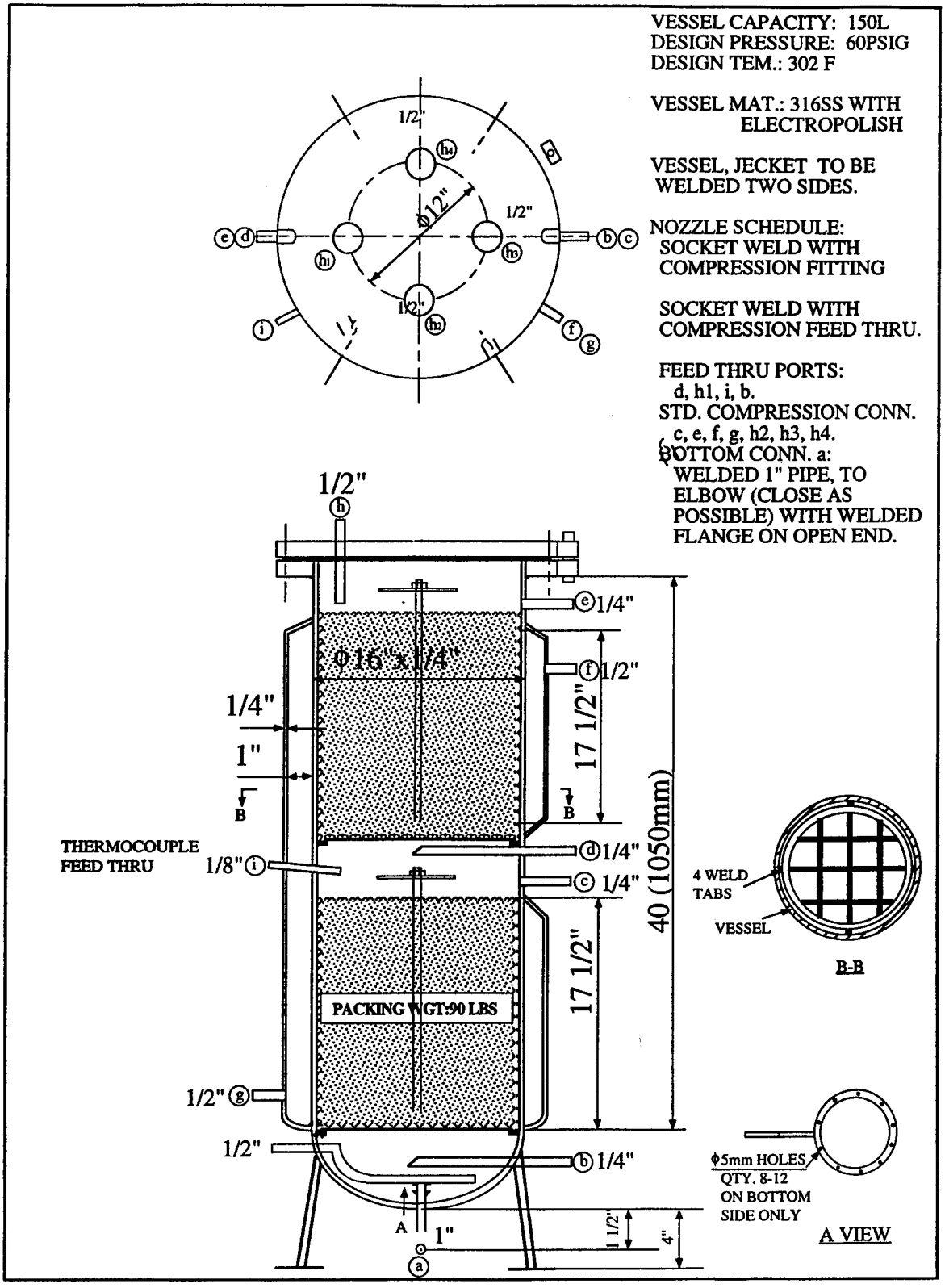


Figure A2. Schematic Diagram of 150-liter Fibrous-Bed Bioreactor.

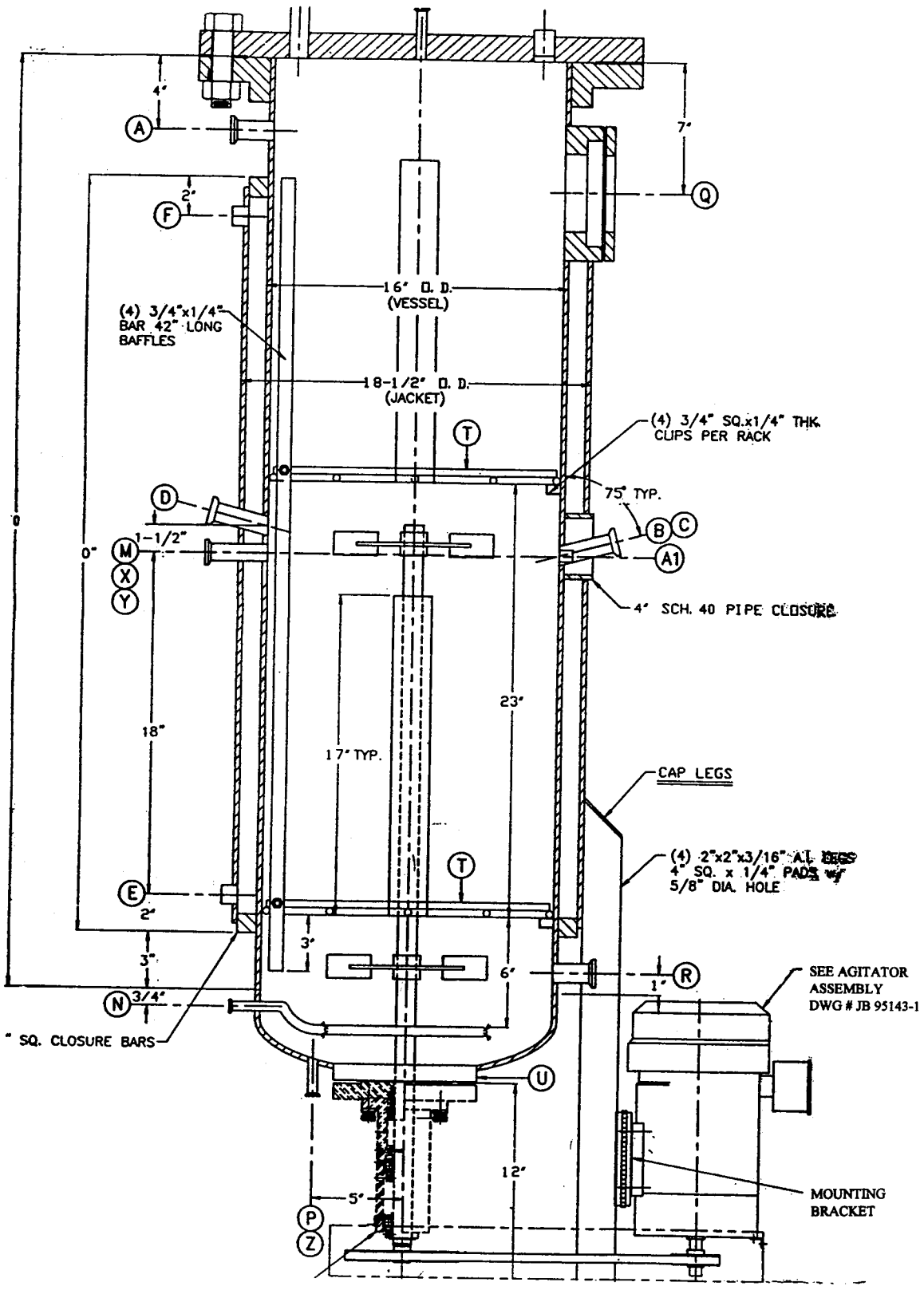


Figure A3. Detailed Engineering Design of 150-liter Fibrous-Bed Bioreactor.

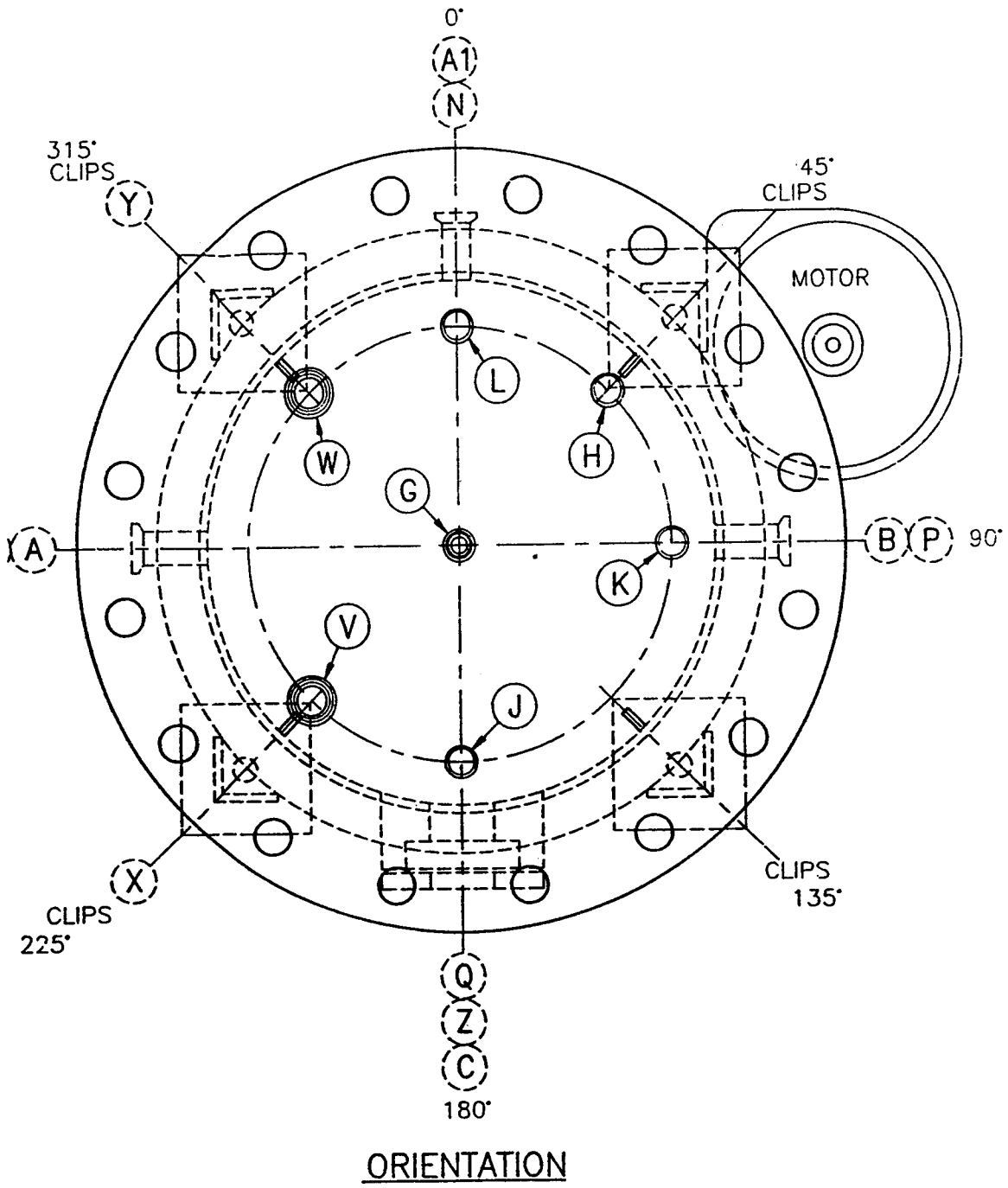


Figure A4. Engineering Design of Top Plate of 150-liter Fibrous-Bed Bioreactor.

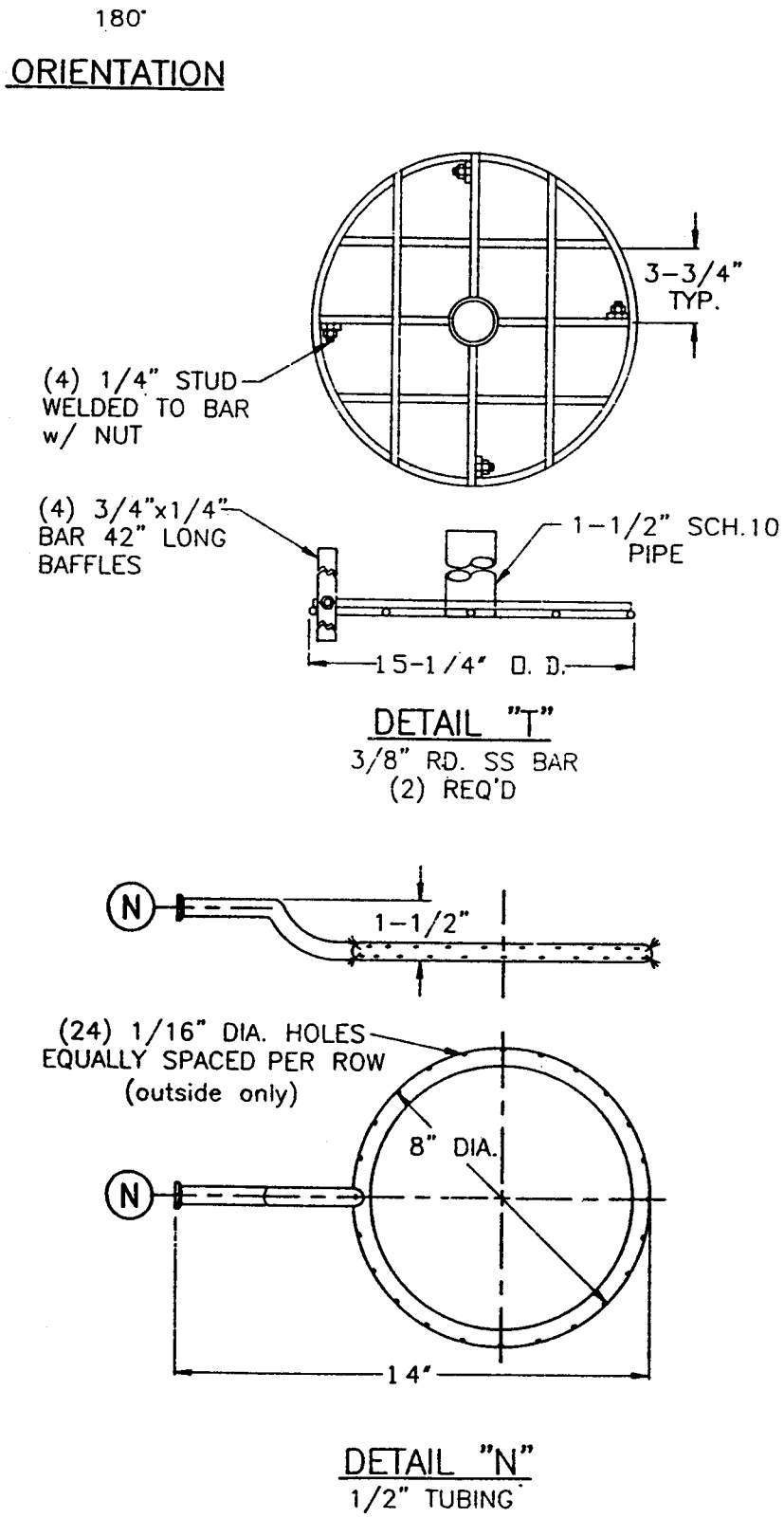


Figure A5. Engineering Design of Air Distributor of 150-liter Fibrous-Bed Bioreactor.

DESIGN DATA

1. VESSEL MATERIAL	ALLOWABLE STRESS	TYPE	THK.
TOP X.RIGHT__.HEAD: SA-182-316	18,480	BLIND	150#
BOTTOM X.LEFT__.HEAD: SA-240-316	18,480	F & D	1/4" nom.
SHELL: SA-240-316	18,480	PLATE	1/4" nom.
BODY FLANGE: SA-182-316	18,480	R.F.S.O.	150#
1a. JACKET MATERIAL			
TOP__.RIGHT__.HEAD:			
BOTTOM__.LEFT__.HEAD:			
SHELL: SA-240-316	18,480	PLATE	1/4" nom.
BOLTS/NUTS: SA-193-G5 or B7 / SA-194-2H			

2. VESSEL TO BE BUILT & LABELED ACCORDING TO ASME CODE SECTION VIII, DIVISION 1, YES X. NO__. 95 EDITION, NO ADDENDA

	VESSEL	JACKET		
DESIGN PRESSURE :	60 psi	60 psi	CODE STAMP :	-U-
TEST PRESSURE :	110 psi	110 psi	CORR. ALLOW. :	None
EXT. PRESSURE :	60 psi	---	X-RAY REQ'D :	None
DESIGN TEMP. :	280° F	280° F	HEAT TREAT :	None
MDMT :	-20° F	-20° F	CHARPY IMPACT :	None

OTHER :

3. INTERIOR:
MEDIUM POLISH (AISI #4) 180 GRIT w/ LIGHT BUFF - 32-63 Ra (micro-inch).

4. EXTERIOR: CLEAN

5. NOTES:

5a. ALL BOLTS TO STRADDLE NATURAL C/L OF FLANGES, VESSEL NOT DESIGNED FOR PRE-MADE PIPING. ALL FITTINGS TO BE PROTECTED FOR SHIPMENT. TOLERANCES: SHELL DIA. (OUT OF ROUNDNESS) +/- 1%, FLANGE LEVEL +/- 1%.

5b. CUSTOMER HAS THE RESPONSIBILITY TO VERIFY ALL DIMENSIONS ON THIS DRAWING.

5c. VERTICAL & HORIZONTAL TANKS WITH LEGS OR SADDLES MAY REQUIRE SHIMMING & GROUTING DURING INSTALLATION.

5d. DEBURR AND BREAK ALL SHARP EDGES.

5e. GASKETS SUPPLIED BY OTHERS.

Figure A6. Engineering Design Data of 150-liter Fibrous-Bed Bioreactor (a).

NOZZLES & CONNECTIONS

MARK	SIZE	RATING	TYPE	MATERIAL	NOTES/SERVICE
A	1"	SANITARY	FERRULE	316SS	PRODUCT
B	1"	SANITARY	FERRULE	316SS	PRODUCT
C	1"	SANITARY	FERRULE	316SS	PRODUCT
D	1"	SANITARY	FERRULE	316SS	PRODUCT
E	1"	150#	NPT	SA-182-316	JACKET INLET
F	1/2"	150#	NPT	SA-182-316	JACKET OUTLET
G	1/2"	SANITARY	FERRULE	316SS	PRODUCT
H	1/2"	150#	NPT	SA-182-316	THERMOCOUPLE
J	1/2"	150#	NPT	SA-182-316	PRESSURE GAGE
K	1/4"	150#	NPT	SA-182-316	THERMOMETER
L	1/2"	150#	NPT	SA-182-316	RELIEF VALVE
M	1/2"	SANITARY	FERRULE	316SS	PRODUCT
N	1/2"	SANITARY	FERRULE	316SS	PRODUCT
P	1"	SANITARY	FERRULE	316SS	PRODUCT OUTLET
Q	2"x4"	----	----	316SS	SIGHT WINDOW
R	1"	SANITARY	FERRULE	316SS	PRODUCT
U	3"	150#	PAD FLG.	SA-182-316	AGITATOR
V	1"	SANITARY	FERRULE	316SS	PRODUCT
W	1"	SANITARY	FERRULE	316SS	PRODUCT
X	1"	SANITARY	FERRULE	316SS	PRODUCT
Y	1"	SANITARY	FERRULE	316SS	PRODUCT
Z	1/2"	SANITARY	FERRULE	316SS	PRODUCT FEED
A1	1/4"	150#	NPT	SA-182-316	PRODUCT

BFC
Buckeye Fabricating Co.
245 Pioneer Blvd. Springboro, Ohio 45066
Phone: 513 746-9822 Fax: 513 746-9823

VESSEL DESCRIPTION: 150 LITER STAINLESS STEEL JACKETED TANK				
CUSTOMER: OSU RESEARCH FOUNDATION				P.O. # RF-713768
QTY. 1	DATE: 1-15-96	SCALE: NTS	DR. MJ	APPROV. MB
DRAWING # 23219				REV. 0

Figure A7. Engineering Design Data of 150-liter Fibrous-Bed Bioreactor (b).

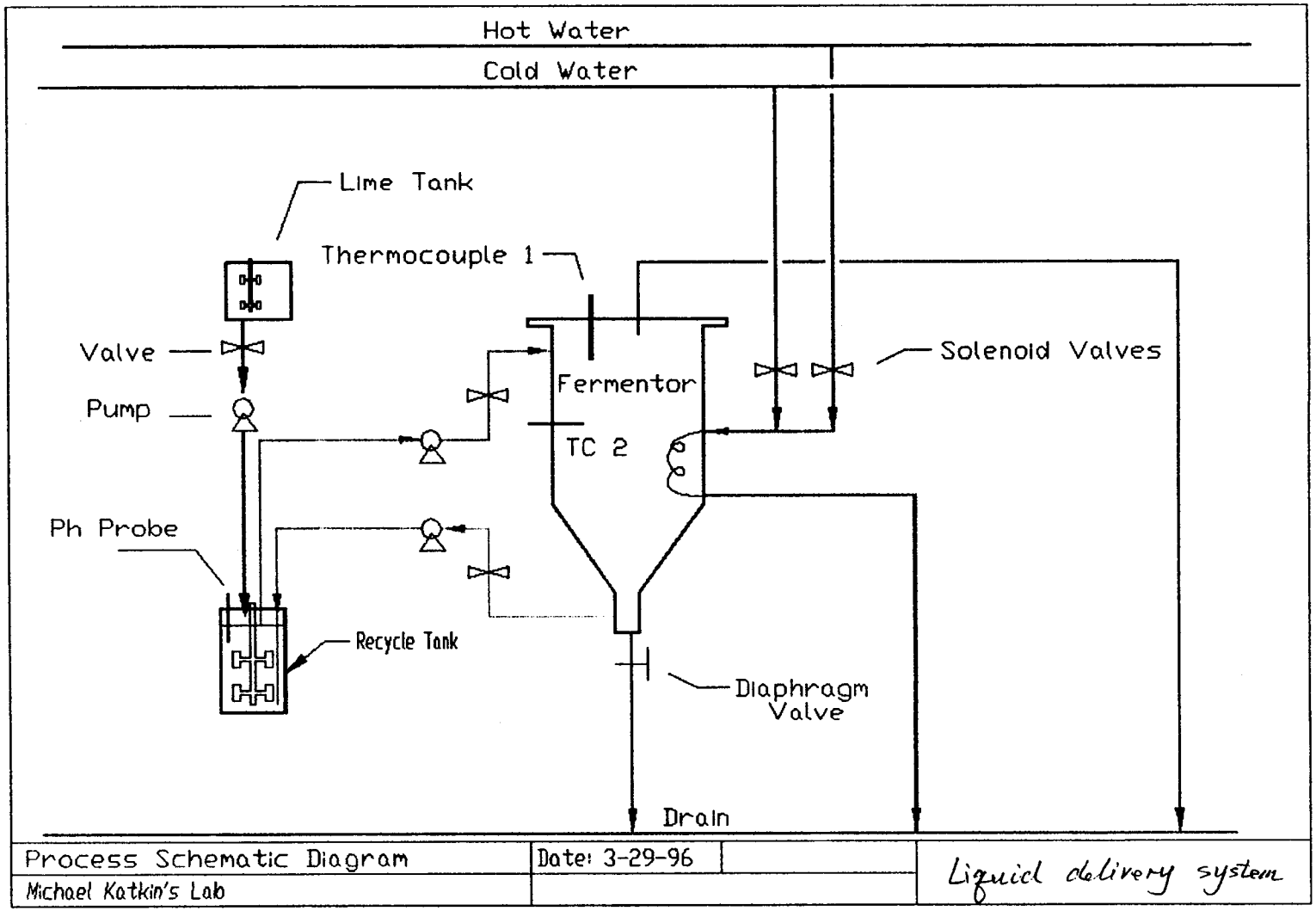


Figure A8. Schematic Diagram of Parameter Control Loops of 150-liter Fibrous-Bed Bioreactor.

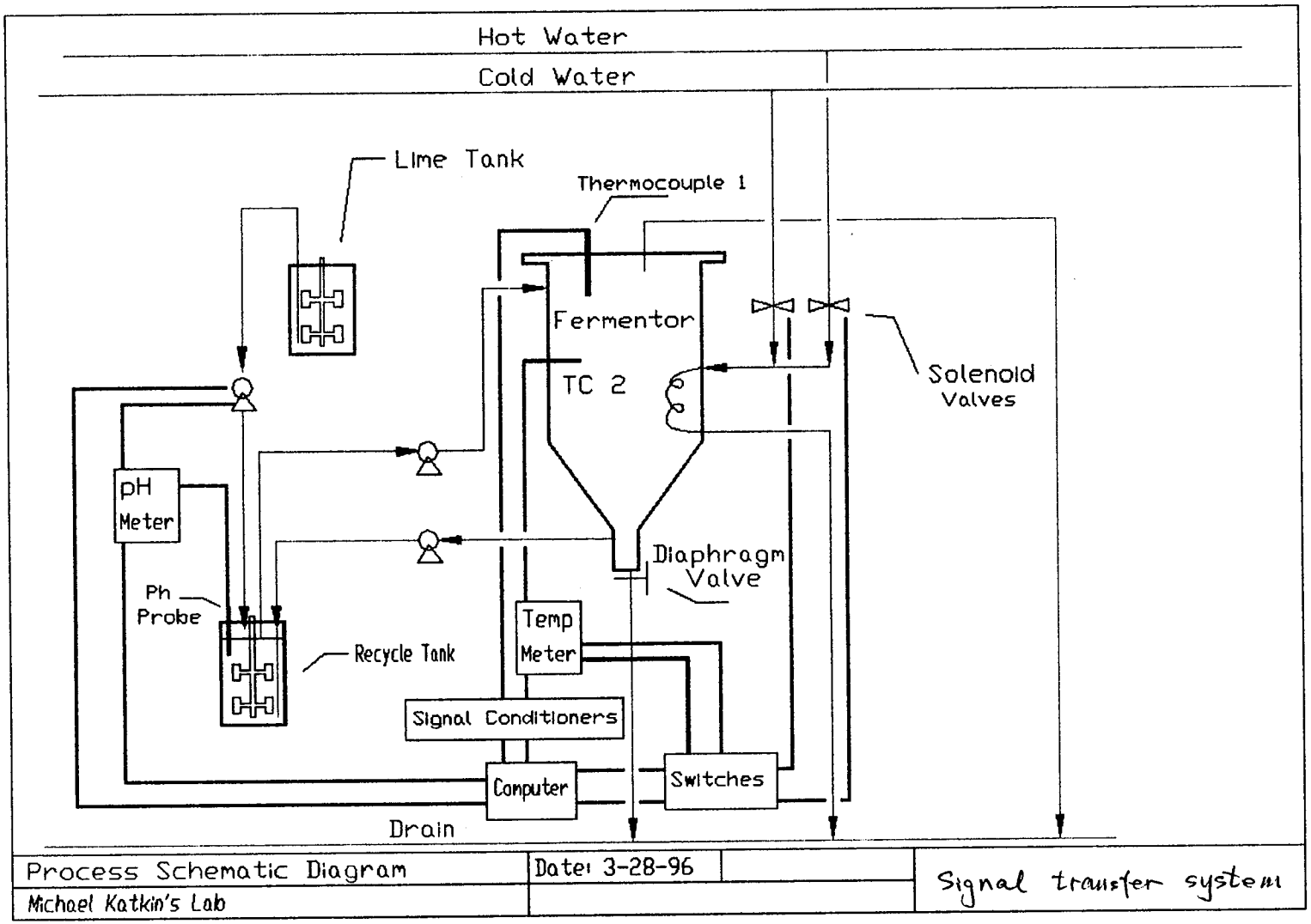


Figure A9. P&I Diagram of 150-liter Fibrous-Bed Bioreactor.

Part #	Description	Company	Model #	Quantity
	SPST Relay	Caydom	D1225	2
	Solenoid Valves	Automatic Switch Co.	8263A210	2
	Temp. Meter	Atunes Control	H-89601-02	1
	Ph Controller	Cole Palmer	5656-05	1
	Agitator	Glass Col	099D HST20N	2
	Recycle Pump	Masterflex	7549-32	1
	Lime Pump	Masterflex	7521-40	1
	Signal Conditioner	Metrabyte	MB47-T-07	1
	Computer	Laser		1
	Analog Input	Keithly Metrabyte	Das 8	1
	Analog Output	Keithly Metrabyte	DDA 6	1

Figure A10. List of Parts Used in Construction of the Computer Controller and Associated Control-Loop Components for 150-liter Fibrous-Bed Bioreactor.

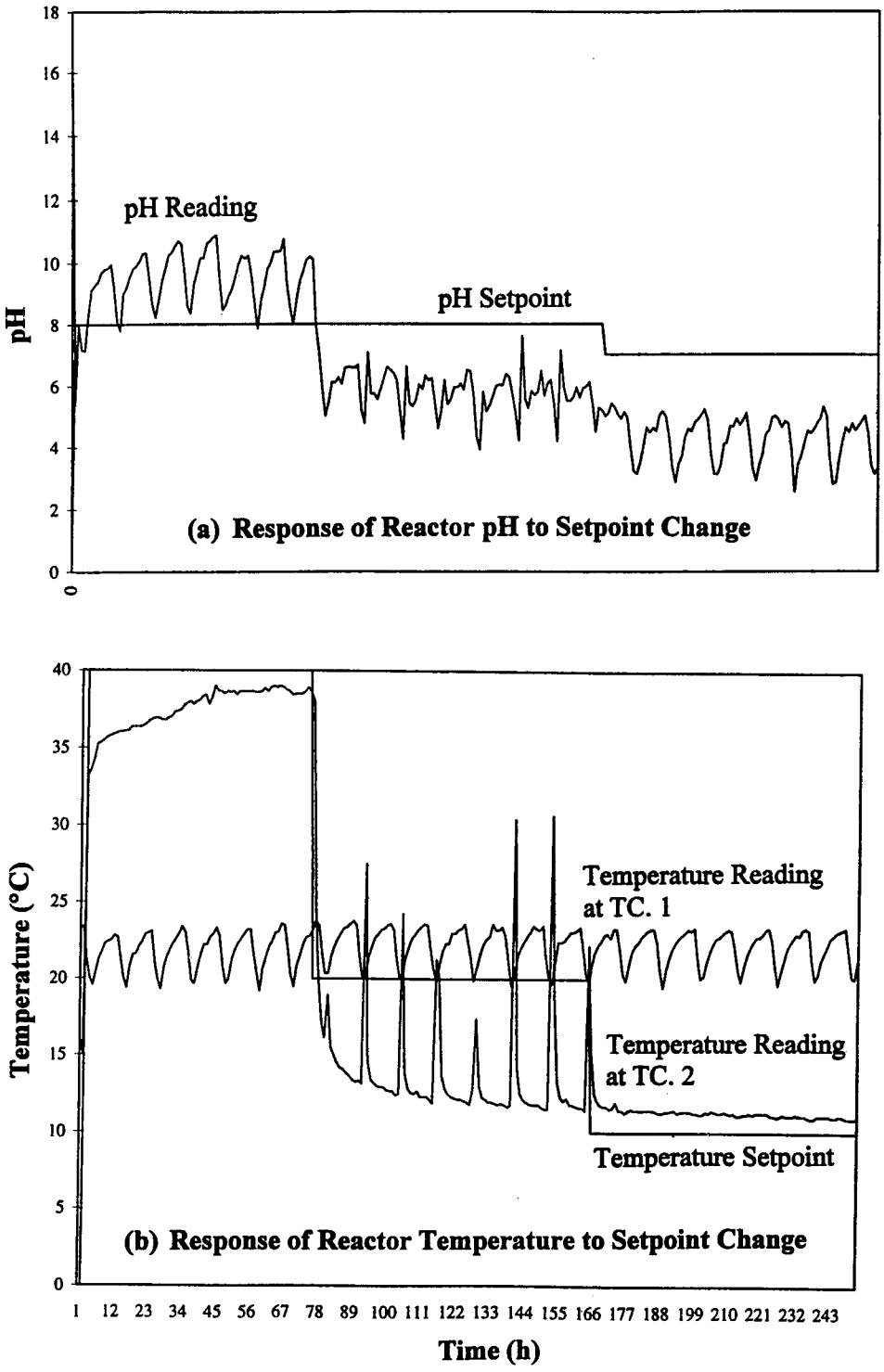


Figure A11. Responses of 150-liter Fibrous-Bed Bioreactor to Controlled pH (a) and Temperature (b); (TC = thermocouple).

APPENDIX B. EXTRACTION KARR COLUMN

The pilot-scale extraction Karr column (Figure B1) used in this work was designed and supplied by Glitsch Technology Corporation. Detailed engineering design is shown in the schematic drawing (Figure B2) and the part list (Figure B3).

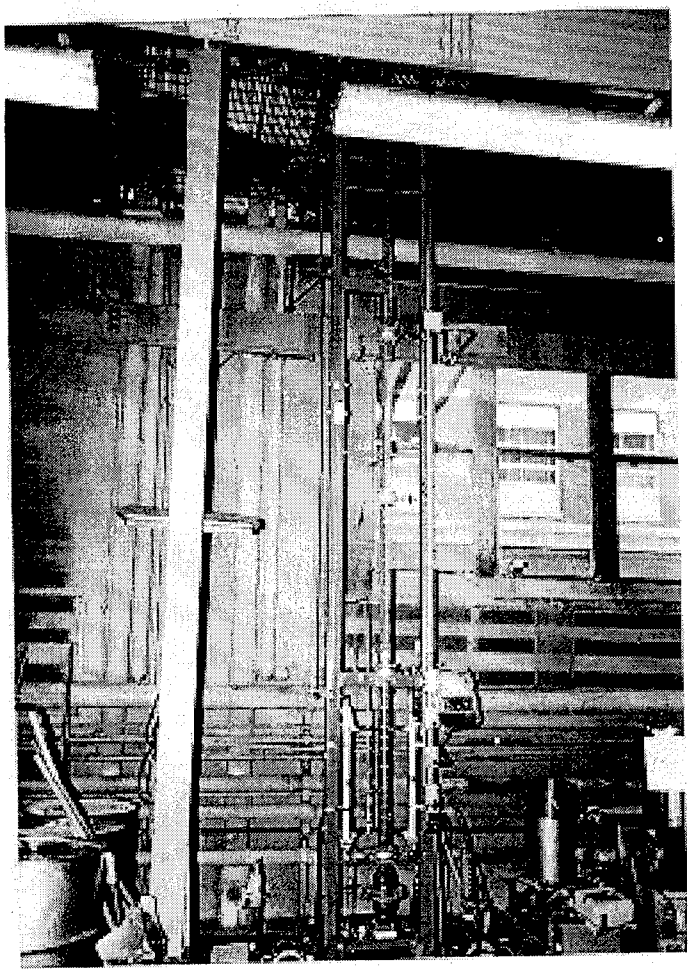
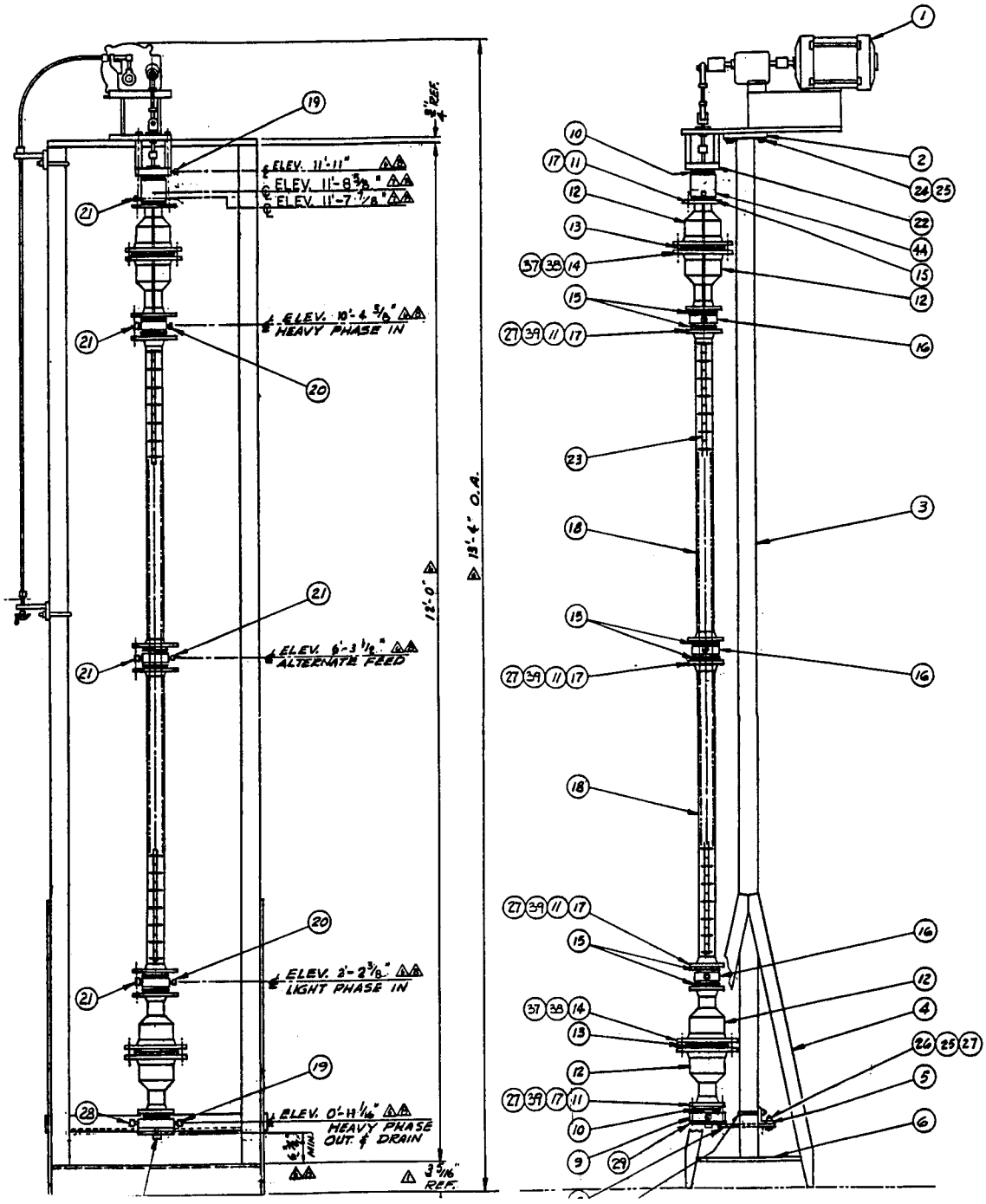
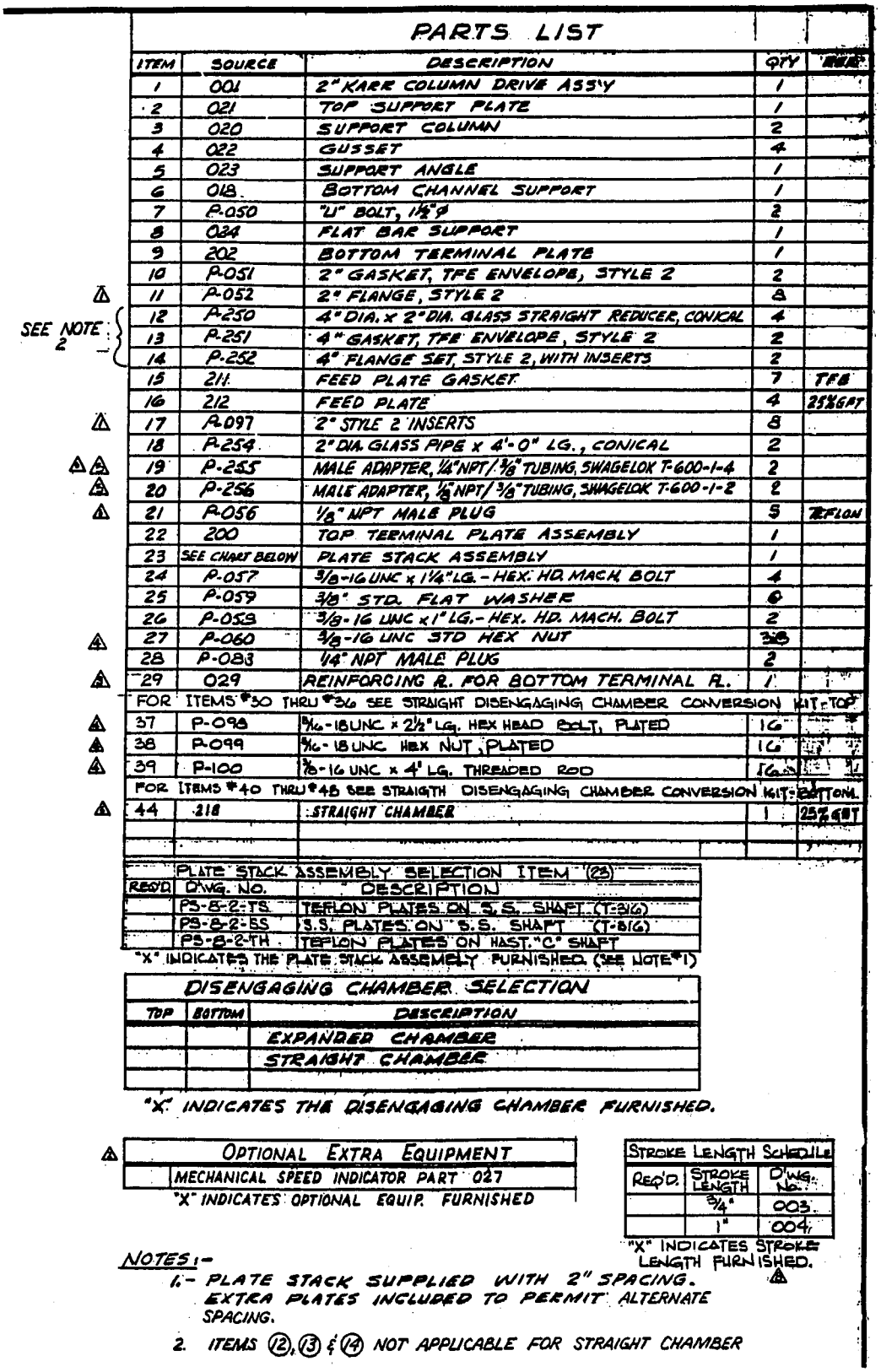


Figure B1. A 16-ft Karr Column (2-in Diameter) Used in Extraction Pilot Plant.



(The scale shown in the drawing is not the same as the one used in this study)

Figure B2. Detailed Schematic of the Karr Column.



PARTS LIST

ITEM	SOURCE	DESCRIPTION	QTY	REMARKS
1	001	2" KARR COLUMN DRIVE ASS'Y	1	
2	021	TOP SUPPORT PLATE	1	
3	020	SUPPORT COLUMN	2	
4	022	GUSSET	4	
5	023	SUPPORT ANGLE	1	
6	018	BOTTOM CHANNEL SUPPORT	1	
7	P-050	"U" BOLT, 1½"ø	2	
8	024	FLAT BAR SUPPORT	1	
9	202	BOTTOM TERMINAL PLATE	1	
10	P-051	2" GASKET, TFE ENVELOPE, STYLE 2	2	
11	P-052	2" FLANGE, STYLE 2	8	
12	P-250	4" DIA. x 2" DIA. GLASS STRAIGHT REDUCER, CONICAL	4	
13	P-251	4" GASKET, TFE ENVELOPE, STYLE 2	2	
14	P-252	4" FLANGE SET, STYLE 2, WITH INSERTS	2	
15	211	FEED PLATE GASKET	7	TFE
16	212	FEED PLATE	4	25%GAT
17	P-097	2" STYLE 2 INSERTS	8	
18	P-254	2" DIA. GLASS PIPE x 4'-0" LG., CONICAL	2	
19	P-255	MALE ADAPTER, ¼"NPT/⅜" TUBING, SWAGELOK T-600-1-4	2	
20	P-256	MALE ADAPTER, ⅛"NPT/⅜" TUBING, SWAGELOK T-600-1-2	2	
21	P-056	⅛" NPT MALE PLUG	5	TEFLON
22	200	TOP TERMINAL PLATE ASSEMBLY	1	
23	SEE CHART BELOW	PLATE STACK ASSEMBLY	1	
24	P-057	⅜-16 UNC x 1¼" LG. - HEX. HD. MACH. BOLT	4	
25	P-059	⅜" STD. FLAT WASHER	6	
26	P-058	⅜-16 UNC x 1" LG. - HEX. HD. MACH. BOLT	2	
27	P-060	⅜-16 UNC STD HEX NUT	36	
28	P-083	¼" NPT MALE PLUG	2	
29	029	REINFORCING R. FOR BOTTOM TERMINAL PL.	1	
FOR ITEMS #30 THRU #36 SEE STRAIGHT DISENGAGING CHAMBER CONVERSION KIT - TOP				
37	P-098	⅝-18 UNC x 2½" LG. HEX HEAD BOLT, PLATED	16	
38	P-099	⅝-18 UNC HEX NUT, PLATED	16	
39	P-100	⅝-16 UNC x 4' LG. THREADED ROD	16	
FOR ITEMS #40 THRU #48 SEE STRAIGTH DISENGAGING CHAMBER CONVERSION KIT - BOTTOM.				
44	218	STRAIGHT CHAMBER	1	25%GBT

PLATE STACK ASSEMBLY SELECTION ITEM (23)		
REQ'D	D'WG. NO.	DESCRIPTION
	PS-8-2-TS	TEFLON PLATES ON S.S. SHAFT (T-316)
	PS-8-2-SS	S.S. PLATES ON S.S. SHAFT (T-316)
	PS-8-2-TH	TEFLON PLATES ON HAST. "C" SHAFT

"X" INDICATES THE PLATE STACK ASSEMBLY FURNISHED. (SEE NOTE #1)

DISENGAGING CHAMBER SELECTION		
TOP	BOTTOM	DESCRIPTION
		EXPANDED CHAMBER
		STRAIGHT CHAMBER

"X" INDICATES THE DISENGAGING CHAMBER FURNISHED.

OPTIONAL EXTRA EQUIPMENT	
	MECHANICAL SPEED INDICATOR PART 027

"X" INDICATES OPTIONAL EQUIP. FURNISHED

STROKE LENGTH SCHEDULE		
REQ'D.	STROKE LENGTH	D'WG. NO.
	¾"	003
	1"	004

"X" INDICATES STROKE LENGTH FURNISHED.

NOTES:-

1. PLATE STACK SUPPLIED WITH 2" SPACING. EXTRA PLATES INCLUDED TO PERMIT ALTERNATE SPACING.
2. ITEMS (12), (13) & (14) NOT APPLICABLE FOR STRAIGHT CHAMBER

Figure B3. Part List for the Karr Column.

APPENDIX C. MICROFILTRATION SYSTEM DESIGN

The cheese whey permeate used in this study was from an off-site dairy plant. It was necessary, especially at the initial stage of bioreactor start-up, to pre-treat and sterilize the whey by microfiltration. A membrane filtration process consisting of a prefiltration and a microfiltration system was designed (Figure C1). The pre-filter (with 2-μm membrane pore size) was used to remove micron-sized soid particulates from whey permeate to protect the microfiltration membrane (0.2-μm pore size). The microfiltration system contained a spiral-wound membrane (Figure C2) and had the following features:

- In-line cleaning facility for membrane cleaning and storage.
- A treatment capacity up to 20 gal per hour.
- A treatment efficiency of more than 99.9%.
- Flexible applications for microfiltration, ultrafiltration and nanofiltration.

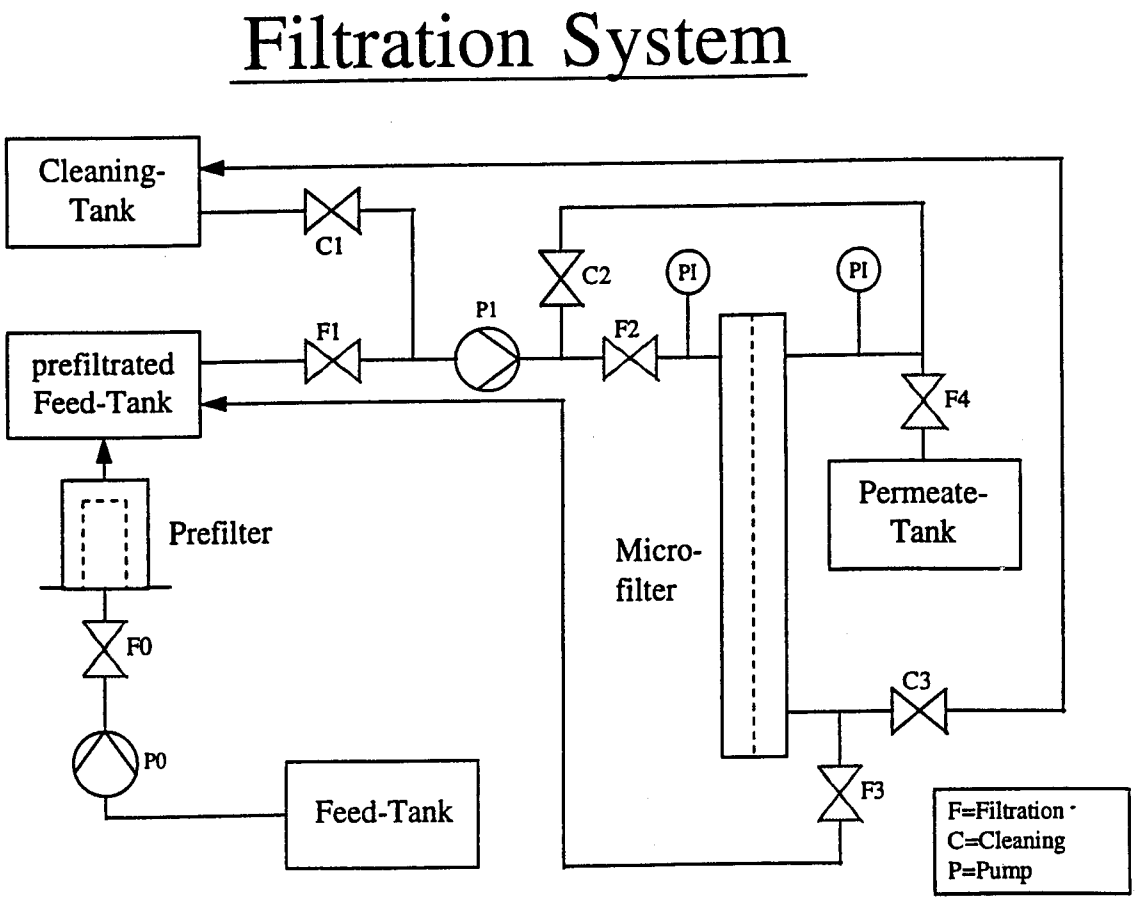
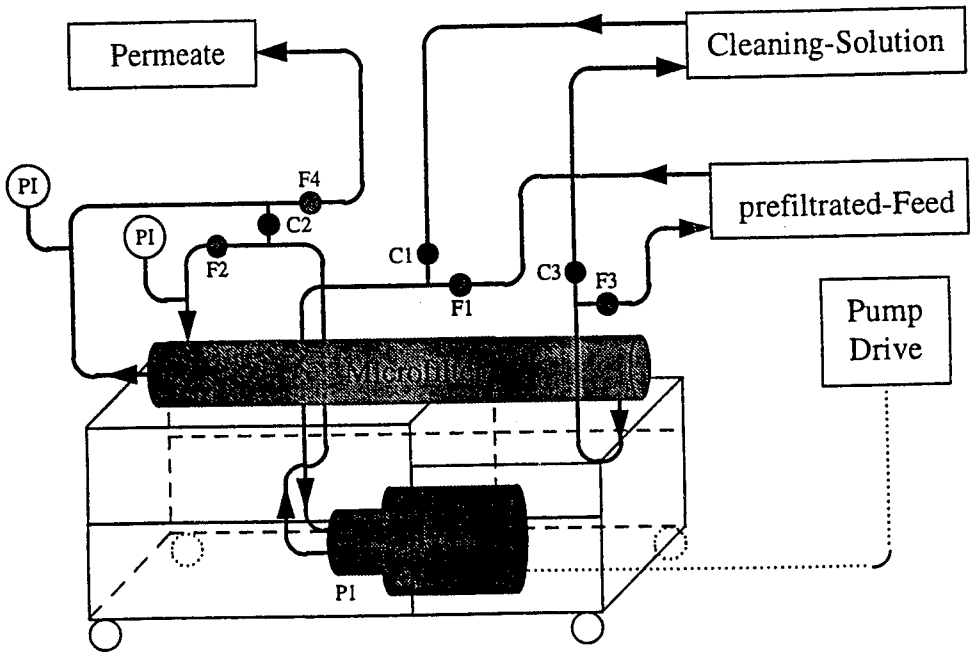


Figure C1. The Filtration System Used for Sterilization of Whey Permeate.

Microfiltration



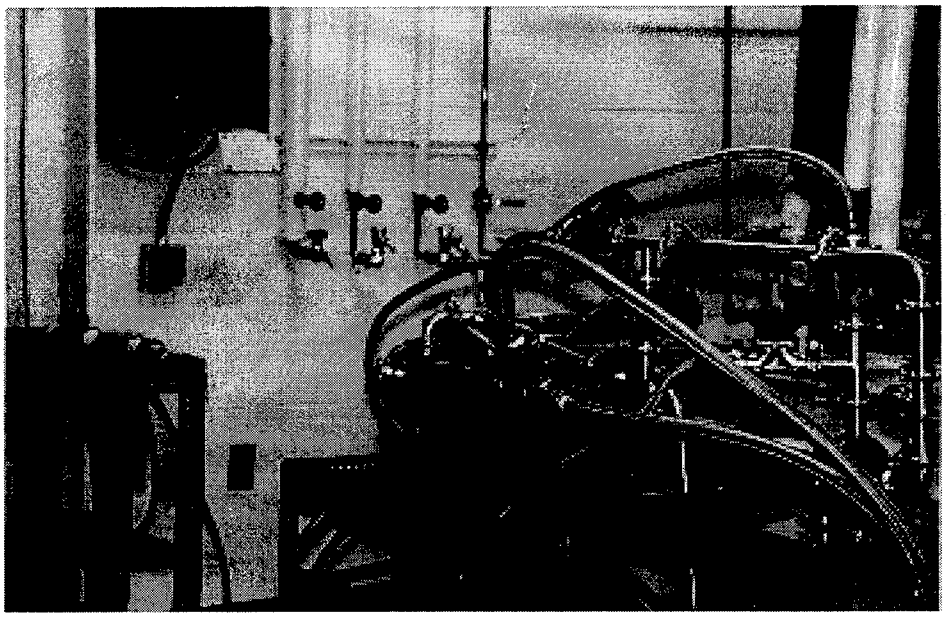


Figure C2. Microfiltration System in the Fermentation Pilot Plant.

BIBLIOGRAPHY

Aeschlimann, A., L. Distasi, and U. Vonstockar (1990). Continuous production of lactic acid from whey permeate by *Lactobacillus helveticus* in two chemostats in series. *Enz. Microb. Technol.*, 12:926-932.

Althouse, J.W. and L.L. Tavlarides (1992). Analysis of organic extractant systems for acetic acid removal for calcium magnesium acetate production. *Ind. Eng. Chem. Res.*, 31:1971-1981.

Andreesen, J.R., G. Gottschalk, and H.G. Schlegel (1970). *Clostridium formicoaceticum* nov. spec. Isolation, description, and distinction from *C. aceticum* and *C. thermoaceticum. Arch. Microbiol.*, 72:154-174.

Balch, W.E., S. Schoberth, R.S. Tanner, and R.S. Wolfe (1977). Acetobacterium, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. *Int. Syst. Bacteriol.*, 27:355-361.

Bar, R. and J.L. Gainer (1987). Acid fermentation in water-organic solvent two-liquid phase systems. *Biotechnol. Progress*, 3:109.

Basu, R. and K.K. Sirkar (1991). Hollow fiber contained liquid membrane separation of citric acid. *AIChE J.*, 37:383-393.

Basu, R. and K.K. Sirkar (1992). Citric acid extraction with microporous hollow fibers. *Solvent Extraction and Ion Exchange*, 10:119-143.

Braun, M., F. Mayer, and G. Gottschalk (1981). *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Arch. Microbiol.*, 128:288-294.

Brumm, P.J. (1988). Fermentation of single and mixed substrate by the parent and an acid-tolerant, mutant strain of *Clostridium thermoaceticum. Biotechnol. Bioeng.*, 32:444-450.

Bryan, W.L. (1992). "Research to reduce the cost of calcium magnesium acetate." In *Chemical Deicers and the Environment*, F.M. D'Itri (ed.), Lewis Publishers, Boca Raton, Chapter 18.

Busche, R.M., E.J. Shimshick, and R.A. Yates (1982). Recovery of acetic acid from dilute acetate solution. *Biotechnol. Bioeng. Symp.*, 12: 249-262.

Chen, R. and Y.Y. Lee (1997). Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass. *Appl. Biochem. Biotechnol.*, 63-65: 435-448.

Chollar, B.H. (1984). Federal Highway Administration research on calcium magnesium acetate - an alternative deicer. *Public Roads*, 47:113.

Compere, A.L. and W.L. Griffith (1975). Fermentation of waste materials to produce industrial intermediates. *Dev. Ind. Microbiol.* 17:241.

Daugulis, A.J. (1988). Integrated reaction and product recovery in bioreactor systems. *Biotechnol. Progress*, 4:113.

DeSouza, B.L. and D.L. Wise (1991). "Process evaluation of CMA production from woody biomass." In *Calcium Magnesium Acetate, An Emerging Bulk Chemical for Environmental Applications*, D.L. Wise, Y.A. Levendis, and M. Metghalchi (eds.), Chapter 17, Elsevier, Amsterdam, Netherlands.

Detroy, R.W. (1981). "Bioconversion of agricultural biomass to organic chemicals." In *Organic Chemicals from Biomass*, I.S. Goldstein (ed.) CRC Press, Boca Raton, Chapter 3.

D'Itri, F.M. (1992). Chemical Deicers and the Environment, Lewis Publishers, Boca Raton.

Dynatech Scientific, Inc. (1988). Calcium magnesium acetate production and cost reduction. Final report to New York State Energy Research and Development Authority (Report 88-7).

Ebner, H. (1981). "Vinegar." In *Industrial Microbiology*, Prescott and C.G. Dunn (eds.), 4th ed., McGraw-Hill Book Co., Inc., Chapter 18.

Ebner, H. and H. Follmann (1983). "Acetic acid." In *Biotechnology*, H.J. Rehm and G. Reed (eds.), Vol. 3, Verlay Chemie GmbH, Weinheim, Chapter 3b.

Eichler, B. and B. Schink (1984). Oxidation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe. *Arch. Microbiol.*, 140:147-152.

Evans, P.J. and H.Y. Wang (1990). Effects of extractive fermentation on butyric acid production by *Clostridium acetobutylicum*. *Appl. Microbiol. Biotechnol.*, 32:393-397.

Eyal, A.M. and R. Canari (1995). pH Dependence of carboxylic and mineral acid extraction by amine based extractants: effects of pK_a, amine basicity, and diluent properties. *Ind. Eng. Chem. Res.*, 34: 1789-798.

Fahrim, M.A., A. Qader, and M.A. Hughes (1992). Extraction equilibria of acetic acid and propionic acid from dilute aqueous solution by several solvent. *Sep. Sci. Technol.*, 27:1809-1821.

Fritzsche, C.J. (1992). Nonpoint source pollution, Calcium Magnesium Acetate deicer, an effective alternative for salt-sensitive areas. *Water Environment Technol.*, January 1992, 44-51.

Ghose, T.K. and A. Bhadra (1985). "Acetic acid." In *Comprehensive Biotechnology*, M. Moo-Young (ed), Vol. 3, Pergamon Press, New York, Chapter 36.

Harrach, N. and J. Wyatt (1990). Fine tuning CMA for corrosion control. *Public Works*, July, pp. 40-41.

Harper, W.J. and C.W. Hall (1976). Dairy Technology and Engineering. AVI Publishing, Westport, p. 211.

Hatzinibolaou, D. and H. Wang (1992), Extractive fermentation systems for organic acid production. *Can. J. Chem. Eng.*, 70:543-552.

Helsel, R.W. (1977). Removing carboxylic acids from aqueous wastes. *Chem. Eng. Prog.*, 73(5):55-59.

Hiatt, G.F.S., N.A. George, J.R. Cushman, L.C. Griefis, and G.A. Rausina (1988). Calcium magnesium acetate: comparative toxicity tests and an industrial hygiene site investigation. *Transportation Resch. Record*, 1157:20-26.

Hobman, P.G. (1984). Review of processes and products for utilization of lactose in deproteinated milk serum. *J. Dairy Sci.*, 67:2630.

Honda, H., Y. Toyama, H. Takahashi, T. Nakazeko, and T. Kobayashi (1995). Effective lactic acid production by two-stage extractive fermentation. *J. Ferment. Bioeng.*, 79:589-593.

Honda, H., T. Mano, M. Taya, K. Shimizu, M. Matsubara, and T. Kobayashi (1987). A general framework for the assessment of extractive fermentations. *Chem. Eng. Sci.*, 42:493-498.

Huang, Y. (1998). Process and metabolic engineering for enhanced propionic and acetic acid fermentations. Ph.D. Thesis, The Ohio State University, Columbus, Ohio.

Huang, Y. and S.T. Yang (1998). Acetate production from whey lactose using co-immobilized cells of homolactic and homoacetic bacteria in a fibrous-bed bioreactor. *Biotechnol. Bioeng.*, 60:498-507.

Huang, Y.L., K. Mann, J.M. Novak, and S.T. Yang (1998). Acetic acid production from fructose by *Clostridium formicoaceticum* in a fibrous-bed bioreactor. *Biotechnol. Prog.*, 14:800-806.

Hudson, L.R. (1987). Calcium magnesium acetate (CMA) from low grade biomass. IGT conference on "Energy from Biomass and Wastes XI," Orlando, Florida.

Ito, L., H. Sota, H. Honda, K. Shimizu, and T. Kobayashi (1991). Efficient acetic acid production by repeated fed-batch fermentation using two fermentors. *Appl. Microbiol. Biotechnol.*, 36:295-299.

Jagirdar, G.C. and M.M. Sharma (1980). Recovery and separation of mixtures of organic acids from dilute aqueous solutions. *J. Sep. Proc. Technol.*, 1(2):40-43.

Jin, Z-W. (1997). Continuous extractive fermentation using hollow fiber membrane extractors for enhanced propionate production, Ph.D. Thesis, The Ohio State University, Columbus, Ohio.

Jin, Z. and S.T. Yang (1998). Extractive fermentation for enhanced propionic acid poduction from lactose by *Propionibacterium acidipropionici*. *Biotechnol. Progress*, 14:457-465.

Juang, R.-S. and L.-J. Chen (1996). Analysis of the transport rates of citric acid through a supported liquid membrane containing tri-n-octylamine. *Ind. Eng. Chem. Res.*, 35:1673-1679.

Katzbauer, B., V. Cesi, M. Noradoslawsky, and A. Moser (1995). Extractive lactic acid fermentation using aqueous two-phase systems. *Chem. Biochem. Eng. Q.*, 9(2):79-87.

Kertes, A.S. and C.J. King (1986). Extraction chemistry of fermentation product carboxylic acids. *Botechnol. Bioeng.*, 28:269-282.

Kiani, A., R.R. Bhave, and K.K. Sirkar (1984). Solvent extraction with immobilized interfaces in a microporous hydrophobic membrane. *J. Membrane Sci.*, 20:125-145.

King, C.J. (1992). Amine-based systems for carboxylic acid recovery. *CHEMTECH*, May 1992:285-291.

Kondo, T. and Kondo, M. (1996). Efficient production of acetic acid from glucose in a mixed culture of *Zymomonas mobilis* and *Acetobacter* sp. *J. Fermentation Bioeng.*, 81:42-46.

Kwon, Y. J., R. Kaul, B. Mattiasson (1996). Extractive lactic acid fermentation in poly(ethyleneimine)-based aqueous two-phase system. *Biotechnol. Bioeng.*, 50:280-290.

Leigh, J.A., F. Mayer, and R.S. Wolfe (1981). *Acetogenium kivui*, a new thermophilic hydrogen-oxidizing, acetogenic bacterium. *Arch. Microbiol.*, 129:275-280.

Lewis, V.P. and S.T. Yang (1992). A novel extractive fermentation process for propionate production from whey lactose. *Biotechnol. Progress*, 8:104-110.

Ljungdahl, L.G. (1983). "Formation of acetate using homoacetate fermenting anaerobic bacteria." In *Organic Chemicals from Biomas,* D.L.Wise (ed.), 219-248. Benjamin/Cummings Publishing Co., London.

Ljungdahl, L.G., L.H. Carreira, R.J. Garrison, N.E. Rabek, and J. Weigel (1985). Comparison of three thermophilic acetogenic bacteria for production of calcium-magnesium acetate. *Biotechnol. Bioeng. Symp.*, 15:207-223.

Ljungdahl, L.G., L.H. Carreira, R.J. Garrison, N.E. Rabek, and J. Weigel (1986). CMA manufacture (II): improved bacterial strain for acetate production. Final report, FHWA/RD-86/117.

Marynowski, C.W., J.L. Jones, D. Tuse, and R.L. Boughton (1984). Fermentation as an advantageous route for the production of an acetate salt for roadway deicing. SRI report.

Marynowski, C.W., J.L. Jones, D. Tuse, and R.L. Boughton (1985). Fermentation as an advantageous route for the production of an acetate salt for roadway deicing. *Ind. Eng. Chem. Res. Dev.*, 24:457-465.

Ministry of Transportation of Ontario (1989). CMA - A practical alternative to salt? *Ontario R & D Report*, 1(1):1-4.

Normura, Y., M. Iwahara, and M. Hongo (1988). Acetic acid production by an electrodialysis fermentation method with a computerized control system. *Appl. Environ. Microbiol.*, 54:137-142.

Parekh, S.R. and M. Cheryan (1990a). Acetate production from glucose by *Clostridium thermoaceticum. Process Biochem. International*, 25:117-121.

Parekh, S.R. and M. Cheryan (1990b). Fed-batch fermentation of glucose to acetate by an improved strain of *Clostridium thermoaceticum. Biotechnol. Letters*, 12:861-864.

Parekh, S.R. and M. Cheryan (1991). Production of acetate by mutant strains of *Clostridium thermoaceticum. Appl. Microbiol. Biotechnol.*, 36:384-387.

Parekh, S.R. and M. Cheryan (1994a). High concentrations of acetate with a mutant strain of *C. thermoaceticum. Biotechnol. Letters*, 16:139-142.

Parekh, S.R. and M. Cheryan (1994b). Continuous production of acetate by *Clostridium thermoaceticum* in a cell-recycle membrane bioreactor. *Enzyme Microb. Technol.*, 16:104-109.

Park, Y.S., K. Toda, M. Fukaya, H. Okumura, and Y. Kawamura (1991a). Production of a high concentration acetic acid by *Acetobacter aceti* using a repeated fed-batch culture with cell recycling. *Appl. Microbiol. Biotechnol.*, 35:149-153.

Park, Y.S., M. Fukaya, H. Okumura, Y. Kawamura, and K. Toda (1991b). Production of acetic acid by a repeated batch culture with cell recycle of *Acetobacter aceti*. *Biotechnol. Lett.*, 13(4):271-276.

Peters, M.S. and K.D. Timmerhaus (1991). Plant Design and Economics for Chemical Engineers. 4th edition, McGraw-Hill, New York.

Planas, J., P. Radstrom, F. Tjerneld, and H.B. Hahn (1996). Enhanced production of lactic acid through the use of a novel aqueous two-phase system as an extractive fermentation system. *Appl. Microbiol. Biotechnol.*, 45, 737-743.

Qin, W. and S.T. Yang (1997). Separation of acetic acid and lactic acid in fermentation by solvent extraction using hollow fiber membrane extractor. *AIChE 1997 Ann. Meeting*, Los Angeles, CA.

Reed, W.M., F.A. Keller, F.E. Kite, M.E. Bogdan, and J.S. Ganoung (1987). Development of increased acetic acid tolerance in anaerobic homoacetogens through induced mutagenesis and continuous selection. *Enzyme Microb. Technol.*, 9:117-120.

Ricker, N.L., J.N. Michaels, and C.J. King (1979). Solvent extraction with amines for recovery of acetic acid from dilute aqueous industrial streams. *J. Separ. Proc. Technol.*, 1:36-41.

Reisinger, H., C.J. King (1995). Extraction and sorption of acetic acid at pH above pK_a to form calcium magnesium acetate. *Ind. Eng. Chem. Res.*, 34:845-852.

Roffler, S. R., H.W. Blanch, and C.R. Wilke (1984). In situ recovery of fermentation products. *Trends in Biotechnol.* 2(5):129.

Robinson, R.G. and D.Y. Cha (1985). Controlled pH extraction in separation of weak acids and bases: increasing extraction selectivity in multistage extractions. *Biotechnol. Prog.*, 1:18-25.

San Nicolas, E., W. Qin, and S.T. Yang (1997). An extractive fermentation process for acetate production from biomass. *AIChE 1997 Ann. Meeting*, 1997, Los Angeles, California.

Schink, B. (1984). *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch. Microbiol.*, 137:250-255.

Scholler, C., J.B. Chaudhuri, and D.L. Pyle (1993), Emulsion liquid membrane extraction of lactic acid from aqueous solutions and fermentation broth. *Biotechnol. Bioeng.*, 42:50-58.

Schwartz, R.D. and F.A. Keller, Jr. (1982a). Acetic acid production by *Clostridium thermoaceticum* in pH-controlled batch fermentation at acidic pH. *Appl. Environ. Microbiol.*, 43:1385-1392.

Schwartz, R.D. and F.A. Keller, Jr. (1982b). Isolation of a strain of *Clostridium thermoaceticum* capable of growth and acetic acid production at pH 4.5. *Appl. Environ. Microbiol.*, 43:117-123.

Seevaratnam, S., O. Holst, S. Hjoerleifsdottir, and B. Mattiasson (1991). Extractive bioconversion for lactic acid production using solid sorbent and organic solvent. *Bioprocess Eng.*, 6:35-41.

Sengupta, A., R. Basu, and K.K. Sirkar (1988). Separation of solutes from aqueous solutions by contained liquid membranes. *AIChE J.*, 34:1698-1708.

Slick, D.S. (1988). Effects of calcium magnesium acetate on pavements and motor vehicles. *Transportation Resch. Record*, 1157:27-30.

Shah, M.M. and M. Cheryan (1995). Improvement of productivity in acetic acid fermentation with *Clostridium thermoaceticum*. *Appl. Biochem. Biotechnol.*, 51/52:413-422.

Silva, E.M. and S.T. Yang (1995). Continuous production of lactic acid from acid whey by *Lactobacillus helveticus* in a fibrous-bed bioreactor. *J. Biotechnol.*, 41:59-70.

Solichien, M.S., D. O'Brien, E.G. Hammond, and C.E. Glatz (1995). Membrane-based extractive fermentation to produce propionic and acetic acids: toxicity and mass transfer considerations. *Enz. Microb. Technol.*, 17:23-31.

Srivastava, A., P.K. Roychoudhury, and V. Sahai (1992). Extractive lactic acid fermentation using ion-exchange resin. *Biotechnol. Bioeng.*, 39:607-613.

Srivastava, A., P. K. Roychoudhury, and V. Sahai (1992). Extractive lactic acid fermentation using ion-exchange resin. *Biotechnol. Bioeng.*, 39:607-613.

Starr, J.N. and C. J. King (1992). Water-enhanced solubility of carboxylic acid in organic solvent and its application to extraction process. *Ind. Eng. Chem. Res.*, 31:2572-2579.

Tamada, J.A., A.S. Kertes, and C.J. King (1990). Extraction of carboxylic acids with amine extractants. 1. Equilibria and law of mass action modeling. *Ind. Eng. Chem. Res.*, 29:1319-1326.

Tamada, J.A. and C.J. King (1990). Extraction of carboxylic acids with amine extractants. 3. Effect of temperature, water coextraction, and process considerations. *Ind. Eng. Chem. Res.*, 29:1333-1338.

Tang, I.C. (1986). Effects of pH on acetic acid production from lactose by an anaerobic mixed culture fermentation. Ph.D. Dissertation, Purdue University, West Lafayette, Indiana.

Tang, I.C., S.T. Yang, and M.R. Okos (1988). Acetic acid production from whey lactose by the coculture of *Streptococcus lactis* and *Clostridium formicoaceticum*. *Appl. Microbiol. Biotechnol.*, 28:138-143.

Tang, I.C., M.R. Okos, and S.T. Yang (1989). Effects of pH and acetic acid on homoacetic fermentation of lactate by *Clostridium formicoaceticum*. *Biotechnol. Bioeng.*, 34:1063-1074.

Transportation Research Board (1991). Highway Deicing, Comparing Salt and Calcium Magnesium Acetate, Special Report 235, National Research Council, Washington, D.C.

Trantolo, D.J., J.D. Gresser, D.C. Augenstein, and D.L. Wise (1991). "Calcium magnesium acetate from the bioconversion of residue biomass." In *Calcium Magnesium Acetate, An Emerging Bulk Chemical for Environmental Applications*, D.L. Wise, Y.A. Levendis, and M. Metghalchi (eds.), Elsevier, Amsterdam, Netherlands, Chapter 15.

Trantolo, D.J., J.D. Gresser, D.C. Augenstein, and D.L. Wise (1990). The feasibility of a residue biomass bioconversion process to prepare calcium magnesium acetate deicing salt. *Resources, Conservation and Recycling*, 4:215-232.

U.S. Economic Research Service. July 1989. *Dairy Situation and Outlook Report.* U.S. Department of Agriculture, Washington, D.C.

Wang, D.I.C., R.J. Fleishchaker, and G.Y. Wang (1978). A novel route to the production of acetic acid by fermentation. *AIChE Symp. Ser.* 181, 74:105-110.

Wang, G. and D.I.C. Wang (1984). Elucidation of growth inhibition and acetic acid production by *Clostridium thermoaceticum. Appl. Environ. Microbiol.*, 47:294-298.

Wang, J., P. Liu, and D. Zhou (1994). Extractive fermentation of lactic acid by immobilized *Lactobacillus casei* using ion-exchange resin. *Biotechnol. Tech.*, 8:905-908.

Wang, Z.H. and K.F. Zhao (1995). Kinetics and mass transfer for lactic acid recovered with anion exchange method in fermentation solution, *Biotechnol. Bioeng.*, 47:1-7.

Wardell, J.M. and C.J. King (1978). Solvent equilibria for extraction of carboxylic acids from water. *J. Chem. Eng. Data*, 23(2):144-148.

Weilnhammer, C. and E. Blass (1994), Continuous fermentation with product recovery by in situ extraction. *Chem. Eng. Technol.*, 17:365-373.

Wennersten, R. (1983). The extraction of citric acid from fermentation broth using a solution of a tertiary amine. *J. Chem. Technol. Biotechnol.*, 33B:85-94.

White, S.A. (1987). Evaluation of tertiary and quaternary amines for use as extractants in the production of organic acids by extractive fermentation. M.S. Thesis, The Ohio State University, Columbus, Ohio.

White, S. and S.T. Yang (1987). Effects of pH on the extractive fermentation for organic acid production using acetogenic anaerobes. Paper presented at the ACS National Meeting, New Orleans, LA, Aug. 30-Sept. 4.

Wiegel, J., M. Braun, and G. Gottschalk (1981). *Clostridium thermoautotrophicum* species novum, a thermophile producing acetate from molecular hydrogen and carbon dioxide. *Current Microbiol.*, 5:255-260.

Wiegel, J., L.H. Carreira, R.J. Garrison, N.E. Rabek, and L.G. Ljungdahl (1991). "Calcium magnesium acetate (CMA) manufacture from glucose by fermentation with thermophilic homoacetogenic bacteria." In *Calcium Magnesium Acetate, An Emerging Bulk Chemical for Environmental Applications*, D.L. Wise, Y.A. Levendis, and M. Metghalchi (eds.) Elsevier, Amsterdam, Netherlands, Chapter 16.

Wise, D.L. and D.C. Augenstein (1988). An evaluation of the bioconversion of woody biomass to calcium acetate deicing salt. *Solar Energy*, 41:453-463.

Yabannavar, V.M. and D.I.C. Wang (1991a). Strategies for reducing solvent toxicity in extractive fermentations. *Biotechnol. Bioeng.*, 37:716-722.

Yabannavar, V.M. and D.I.C. Wang (1991b). Extractive fermentation for lactic acid production. *Biotechnol. Bioeng.*, 37:1095-1100.

Yang, S.T. (1984). Kinetic study of defined methane fermentation of lactose. Ph.D. Thesis, Purdue University, West Lafayette, Indiana.

Yang, S.T. (1988). Extractive fermentation for organic acid production using acetogenic anaerobes. Poster paper presented at the 8th International Biotechnology Symposium, Paris, France, July 17-22.

Yang, S.T. (1991). "A novel method to produce road deicer from cheese whey." In *Proceedings of Waste Stream Minimization and Utilization Innovative Concepts - An Experimental Technology Exchange*, R.L. Watts and V.E. Lee (eds.), Washington D.C., April 25-26. Vol. 2, pp. 9.1-9.13.

Yang, S.T. and B.H. Chollar (1996). Production of low-cost acetate deicers from biomass and industrial wastes. *Proceedings for the 4th International Symposium on Snow Removal and Ice Control Technology*, Reno, Nevada, August 12-16, Transportation Research Board, National Research Council; Vol. 1, pp. C-6.

Yang, S.T., I.C. Tang, and M.R. Okos (1987). Kinetics of homoacetic fermentation of lactate by *Clostridium formicoaceticum. Appl. Environ. Microbiol.*, 53:823-827.

Yang, S.T., I.C. Tang, and M.R. Okos (1988). Kinetics and mathematical modeling of homoacetic fermentation of lactate by *Clostridium formicoaceticum. Biotechnol. Bioeng.*, 32:797-802.

Yang, S.T., S.A. White, and S.T. Hsu (1991). Extraction of carboxylic acids with tertiary and quaternary amines: effect of pH. *Ind. Eng. Chem. Res.*, 30:1355-1342.

Yang, S.T., I.C. Tang, and H. Zhu (1992). A novel fermentation process for calcium magnesium acetate (CMA) production from cheese whey. *Appl. Biochem. Biotechnol.*, 34/35:569-583.

Yang, S.T., H. Zhu, V.P. Lewis, and I.C. Tang (1992). Calcium magnesium acetate (CMA) production from whey permeate: process and economic analysis. *Resources Conservation and Recycling*, 7:181-200.

Yang, S.T., H. Zhu, Y. Li, and E.M. Silva (1993). A novel multi-phase bioreactor for fermentations to produce organic acids from dairy wastes. *Proceedings of First Biomass Conference of The Americas*, 8/30 - 9/2, Burlington, Vermont, Vol. 2, pp. 1223-1248.

Yang, S.T., H. Zhu, Y. Li, and G. Hong (1994). Continuous propionate production from whey permeate using a novel fibrous bed bioreactor. *Biotechnol. Bioeng.*, 43:1124-1130.

Yang, S.T., Y. Huang, and G. Hong (1995). A novel recycle batch immobilized cell bioreactor for propionate production from whey lactose. *Biotechnol. Bioeng.*, 45:379-386.

Yang, S.T. and E.M. Silva (1995). Novel products and new technologies for use of a familiar carbohydrate. Milk Lactose, *J. Dairy Sci.*, 78:2563-2583.

Yang, S.T., Z. Jin, and B.H. Chollar (1997). Production of low-cost acetate deicers from biomass and industrial wastes. In *Conference Proceedings 16, Snow Removal and Ice Control Technology*, Transportation Research Board, National Research Council, National Academy Press, Washington D.C., pp. 60-69.

Zhu, H. (1992). A novel immobilized cell fermentation process for calcium magnesium acetate (CMA) production from whey lactose. M.S. Thesis, The Ohio State University, Columbus, Ohio.