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FERMENTATIVE RECYCLING OF LIVESTOCK WASTES AS CRUDE PROTEIN SUPPLEMENTS FOR RUMINANTS

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FERMENTATIVE RECYCLING OF LIVESTOCK WASTES AS CRUDE PROTEIN SUPPLEMENTS FOR RUMINANTS

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ABSTRACT

FERMENTATIVE RECYCLING OF LIVESTOCK WASTES AS CRUDE PROTEIN SUPPLEMENTS FOR RUMINANTS

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The nearly 2 billion tons of livestock wastes produced annually in the United States present a serious pollution hazard to the environment, a financial drain to the livestock industry and a loss of large amounts of potentially usuable nutrients. In an effort to more efficiently utilize these wastes a simple and efficient process for the production of a feed supplement high in crude protein (CP; Nitrogen x 6.25), by bacterial fermentation of feedlot waste filtrate (FLWF) has been developed. When FLWF supplemented with 5% cheese whey powder (CWP; approximately 3.5% lactose) was fermented at 43 C and pH 5.5 for 24 h by the indigeneous microbial flora and concentrated 4 fold a product which contained 55% CP on a wet basis was obtained.

Optimum batch fermentation occured at pH 7.0 and 43 C under these conditions and 90% of the lactose was utilized within 8 h, and the CP content was ∿78% on a dry basis. About 65-70% of the CP was present as non-protein nitrogen in the form of ammonium lactate and ammonium acetate. However, if allowed to ferment 24 h, appreciable amounts of ammonium propionate and ammonium butyrate accumulated while ammonium

lactate concentration decreased. The rates of fermentation and CP content of the product decreased as the pH was lowered. At pH 4.5, 22% of the lactose was unfermented even after 24 h. Fermentation occured most rapidly at 43 C (6 h), less so at 37 C (10 h) and was considerably less efficient at 23 C (21 h) and 50 C (24 h). Fermentation was complete within 20 h when temperature was not controlled, however ambient temperature was 23 C and the temperature within the fermentor rose to 33 C during the peak of fermentation. Lactose concentrations from 3.5 to 8.5% were fermented efficiently.

Continuous fermentation of FLWF at pH 7.0, 43 C and at an optimal retention time (RT) of 1.9 h, the residual lactose concentration was 0.17 g/100 ml (> 96% utilized) and this was 3 to 4 times less than the time required for the batch fermentation of FLWF under identical conditions. The rate of lactose utilization was 19.5 mg/ml/h and was inversely proportional to RT. Organic acid production accounted for > 95% of the lactose utilized. The molar proportion of lactate:acetate:propionate:butyrate at optimal RT was 78:10:3:9. Optimum RTs of FLWF supplemented with 10 and 15% CWP were 7.7 and 10.4 h, respectively; however, the CP content of the product was 17 to 24% lower than FLWF supplemented with 5% CWP. The molar proportions of lactate:acetate:propionate:butyrate were 31:23:18:28 and 31:20:5:44 for FLWF supplemented with 10 and 15% CWP, respectively.

Swine waste filtrate (SWF) and poultry waste filtrate (PWF) were prepared and fermented in a manner identical to

FLWF. Fermentation of unsupplemented wastes for 24 h resulted in little increase in total nitrogen (TN) content although ammonia nitrogen (AN) increased 2.5 and 8.3 fold for SWF and PWF, respectively. Fermented PWF supplemented with 5% CWP contained 1.47 g% TN (62% CP on a dry basis), 93 g% AN and 632 μmoles/ml of organic acids after 8 h. Similarly supplemented and fermented SWF contained 1.19 g% TN (47% CP on a dry basis), 0.63 g% AN, 0.46 g% lactose and 371 μmoles/ml of organic acids.

Fermentation of CWP-supplemented PWF + SWF and PWF + FLWF resulted in products containing 51.6 and 56.7% CP, respectively; AN accounted for 81 and 61% of the CP, respectively. Lactate and acetate accounted for 69 and 21%, respectively, of the total acids produced in CWP-supplemented PWF + FLWF, whereas in corresponding PWF + SWF fermentation, lactate, acetate and propionate were predominant and accounted for 54.5, 24.5 and 15.7% of the total acids produced, respectively.

The fermentative conversion of livestock wastes into potentially valuable ruminant feed supplements as described above, results in reduced pollution to the environment, extends the supply of nitrogenous feed resources and spares large amounts of cereal grains and soybeans for human use.

DEDICATION

To

Barbara, Michael, my family, and

the memory of Henry J. Erdman

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF THE LITERATURE	4
General Characteristics of Livestock Wastes	4
Magnitude	4
Problems	6
Nutritional Potential	6
Livestock Wastes as a Feedstuff - General	8
Recycling Cattle Waste as a Feedstuff	9
Unfermented	9
Aerobic Fermentation	11
Anaerobic Fermentation	13
Recycling Poultry Waste as a Feedstuff	17
Unfermented	17
Aerobic Fermentation	20
Anaerobic Fermentation	21
Insect Biomass Production	23
Recycling Swine Waste as a Feedstuff	24
Unfermented	24
Fermented	27
REFERENCES	30
SECTION 1 (ARTICLE 1) - PRODUCTION OF A RUMINANT PROTEIN SUPPLEMENT BY ANAEROBIC FERMENTATION OF FEEDLOT WASTE FILTRATE	42

Page

SECTION 2 (ARTICLE 2) - OPTIMIZATION OF A BATCH PROCESS FOR THE FERMENTATIVE CONVERSION OF CHEESE WHEY-SUPPLEMENTED CATTLE FEEDLOT WASTE FILTRATE INTO A NITROGENOUS FEED SUPPLEMENT FOR RUMINANTS. 55

SECTION 3 (ARTICLE 3) - A CONTINUOUS FERMENTATION PROCESS FOR RECYCLING FEEDLOT WASTE FILTRATE AS A RUMINANT FEED SUPPLEMENT. 86

SECTION 4 (ARTICLE 4) - PRODUCTION OF NITROGENOUS FEED SUPPLEMENTS FOR RUMINANTS BY BATCH FERMENTATION OF CHEESE WHEY-SUPPLEMENTED POULTRY WASTE, SWINE WASTE, AND CATTLE FEEDLOT WASTE FILTRATES AND MIXTURES OF THESE WASTES. 109

LIST OF TABLES

Table		Page
1	Estimated population and manure production by predominant livestock in the World and United States.	5
2	Proximate analysis of cattle waste.	10
3	Proximate analysis of poultry waste.	18
4	Proximate analysis of swine waste.	25
	SECTION 1	
1	Composition of fiber and FLWF fractions of the FLW.	45
2	Composition of unfermented and fermented FLWF.	48
3	Fermentation of FLWF supplemented with carbohydrate by *Lactobacilli*.	49
4	Fermentation of carbohydrate-supplemented FLWF by natural flora.	50
5	Fermentation of fresh manure supplemented with different carbohydrates.	50
6	VFA composition of product produced by fermentation of FLWF or manure by natural flora.	51
	SECTION 3	
1	Effect of retention time on rate of utilization of lactose and on rates of increase in TN, AN and various organic acids during continuous fermentation of FLWF.	100
2	Effect of supplementation and retention time on concentrations of lactose, TN and AN during fermentation of FLWF.	102
3	Effect of CWP supplementation and retention time of concentration of organic acids produced during the fermentation of FLWF.	104

Table | Page

SECTION 4

1 Composition of unsupplemented PWF, SWF and FLWF before and after fermentation. 117

2 Changes in chemical composition during fermentation of supplemented PWF + FLWF and PWF + SWF. 125

LIST OF FIGURES

Figure		Page
	SECTION 2	
1	Rate of lactose utilization, and rates of increase in AN and TN during the fermentation of FLWF.	62
2	Effect of pH upon TN increase and lactose utilization during fermentation of FLWF. . . .	65
3	Effect of pH on concentrations of organic acids during fermentation of FLWF.	67
4	Effect of temperature on rate of increase in TN during fermentation of FLWF.	70
5	Effect of temperature on organic acids concentration during fermentation of FLWF. . .	72
6	Effect of CWP concentration on rate of increase in TN during fermentation of FLWF. .	75
7	Increase in TN when FLWF from animals fed different rations was fermented.	78
	SECTION 3	
1	Effect of RT on concentrations of lactose, AN, TN and organic acids during continuous fermentation of FLWF.	97
	SECTION 4	
1	Schematic depiction of the fractionation and fermentation procedures used during fermentation.	115
2	Rates of increase in TN, AN and lactose utilization during fermentation of PWF, SWF and FLWF.	120
3	Changes in concentrations of organic acids during fermentation of PWF and SWF.	122

INTRODUCTION

Nearly all plant proteins presently fed to livestock (cattle, chickens and swine) can be consumed by humans. Furthermore, feedlot cattle convert only about 15% of the plant protein consumed into red meat. It is hard therefore, to justify such an inefficient use of our protein resources at a time when protein supplies for human populations are severely limited and expensive in many areas of the world. Consequently, there is a need for developing inexpensive alternative sources of protein for feeding ruminants, utilizing raw materials which are not a part of the human food chain.

Livestock wastes are produced in large quantities. In the United States it is estimated that approximately 1.8×10^{12} kg of livestock wastes are produced annually (69,111). Historically, these wastes have been used as a fertilizer or soil amendent. However, this practice is becoming less practical because of the vast amount of wastes being produced in confined areas, limited land availability and high costs involved in transportation of the wastes to disposal sites. Livestock wastes are also of environmental concern because they can contaminate water resources, are malodorous and provide a breeding site for flies, mosquitoes, pathogenic microorganisms and parasites (7,8,18,40,76,83,136).

Livestock wastes and other agricultural wastes contain large amounts of potentially usable proteins and carbohydrates. For example, livestock wastes were reported to contain approximately 14 - 30% crude protein (CP; Total N x 6.25) and 30 - 50% carbohydrate which is essentially all cellulose and hemicellulose (111). In view of the increasing world-wide demand for protein now and in the forseeable future, it would be desirable to recover the proteins and carbohydrates present in these wastes and recycle them through the ruminant animal to produce red meat for human consumption.

The main objective of the research presented here was to recycle livestock and other agricultural wastes through an ammoniated organic acid fermentation process (41,43,102). In this process readily metabolizable waste carbohydrates are anaerobically fermented to yield organic acids which are neutralized by the addition of ammonia while controlling the temperature and pH of the fermentation. The final nitrogen-rich product is a potentially valuable crude protein supplement for ruminants.

The following four main areas of research were investigated in this study:

1. To determine the feasibility of fermentative conversion of cattle feedlot waste filtrate (FLWF) as a nitrogenous feed supplement for ruminants by an ammoniated organic acid fermentation (43) process;
2. Optimization of the batch fermentation of FLWF into a nitrogenous feed;

3. Study the feasibility of continuously fermenting FLWF;

4. Study the feasibility of fermentative conversion of poultry waste filtrate (PWF), swine waste filtrate, and mixtures of PWF + FLWF, and PWF + swine waste filtrate into nitrogenous feed supplements for ruminants.

REVIEW OF THE LITERATURE

General Characteristics of Livestock Wastes*

Magnitude

Manures from domestic livestock are produced in large amounts. The estimated population and manure production by predominant livestock are presented in Table 1. The estimated World population of predominant livestock used for food and by-products is 8.0×10^9, of which about 72% are chickens, 20% cattle and 8% swine. The estimated domestic livestock population in the United States is 6.5×10^8; chickens, cattle and swine represent 64,26,and 10% of the total, respectively. Although the age of the animal, physiological state, environmental conditions and ration composition greatly influences manure production (118) the average production of total livestock manure from the predominant livestock species worldwide has been estimated to be 1.6×10^{13} kg/yr and in the United States alone about 1.8×10^{12} kg/yr. Cattle account for nearly 93% of the waste produced although they represent only about 23% of the total livestock in numbers.

*For the purposes of this review, livestock wastes are defined as raw manures (feces and urine) combined with feed particles, crop residues, body coverings, broken eggs and soil.

Table 1. Estimated population and manure production by predominant livestock in the World and United States

Animal	Manure Produced[1] (kg/d)	World			United States		
		Animal Numbers[2] (10^7)	Manure Produced (kg/yr x 10^{10})	Distribution[3] (%)	Animal Numbers (10^7)	Manure Produced (kg/yr x 10^{10})	Distribution[3] (%)
Cattle							
Beef	24.6	117.6	1055.9	64.8	12.2	109.5	62.3
Dairy	31.5	40.3	463.3	28.4	4.9	56.3	32.0
Chicken	0.14	577.0	29.5	1.8	41.2	2.1	1.2
Swine	3.4	65.4	81.2	5.0	6.4	7.9	4.5

[1]Average amounts produced per day by an adult animal as reported by Azvedo and Stout (7).

[2]Values as reported by Konigshafer (69).

[3]Distribution of manure among different animal species.

The amount of livestock wastes which are readily collectable is difficult to estimate because of differing animal husbandry techniques adapted in different parts of the World. In the United States, however, it is estimated that nearly 50% of these wastes are produced in intensive animal production systems, such as feedlots and therefore are readily collectable (12).

Problems

Historically, livestock wastes have been used as fertilizer to provide nitrogen, phosphorus and other nutrients to plants. Livestock wastes have also been used as soil amendents to increase organic matter content in soil. However, disposing of livestock wastes on soil as a fertilizer is becoming less practical because of the vast amounts of wastes being produced in confined areas, high disposal costs and continuing decrease in the amount of land available for waste disposal. Livestock wastes are also a serious environmental and public health concern because they can contaminate water resources, are malodorous, pollute the air, and constitute a public health hazard by providing a breeding site for flies, mosquitoes, pathogenic microorganisms and parasites (7,8,18, 26,35,40,76,83,136).

Nutritional Potential

It has been shown that livestock wastes contain potentially valuable nutrients. In the United States it has been estimated that 2.0×10^9 kg of total nitrogen (TN) was

present in livestock wastes produced during 1975 (134). This was approximately equivalent to the TN present in the soybean crop produced during that same year. Since protein is generally the most expensive component of livestock rations, livestock wastes represent a relatively inexpensive crude protein (CP; TN x 6.25) resource which, if properly utilized, would reduce dependence on conventional protein supplements such as soybean meal for milk and meat production.

Sloneker et al. (111) reported the amino acid composition of the protein in fractionated cattle waste to be comparable to soybean meal. Similarly, poultry waste has been shown to be only 29% lower in total animo acids on the average than conventional poultry feeds (36). Poultry waste was also shown to contain high levels of the essential amino acids threonine, valine and lysine. Livestock wastes were also shown to be rich in minerals (34,37,44,88,99,126), B-vitamins (49,50) and energy (9,10,12,25,81,101,115). It can be concluded from the findings presented above that livestock wastes represent vast potentially valuable renewable resources which after suitable processing could be recycled as CP feed supplements to domestic livestock. This would decrease livestock waste disposal costs, reduce air and water pollution problems, lower livestock production costs and increase the supply of cereal grains and soybeans for human consumption.

Livestock Wastes as a Feedstuff - General

The recycling of livestock wastes as a feed ingredient in animal production has been reviewed by Anthony (5,6), Smith (112), Blair and Knight (16,17), Moellers and Vetter (89), Calvert (19), Bhattacharya and Taylor (12), Ichhponani and Lodhi (63) and most recently in the Federal Register (4). Numerous symposia, proceedings and reports which dealt with the processing and utilization of livestock wastes have been published (3,77,78,79,106,107,108,135,137). It is the general consensus that livestock wastes could be satisfactorily substituted for a portion of the conventional animal ration. However, the age of the animal species to be fed, the amount of wastes to be incorporated into the diet and processing techniques used on the wastes were shown to influence animal productivity.

The proximate analysis of cattle waste, poultry waste and swine waste,reported in the literature,is presented in Tables 2,3 and 4, respectively. A typical roughage (alfalfa hay), concentrate energy source (corn grain) and protein source (soybean meal) were also included for comparative purposes in Table 2. The reported compositions of similar livestock waste types show much variation, probably due to varying levels of moisture, ration composition, sample age, physiological state of the animal, environmental effects such as temperature or wind, inclusion of various amounts of foreign matter, variations in analytical procedures and experimental errors.

Recycling Cattle Waste as a Feedstuff

Cattle waste (CW) contain, on the average, 14.7 ± 3.3% CP, 13.6 ± 5.7% ash, 2.3 ± 0.8% ether extract, 37.7 ± 9.7% crude fiber and 30.2 ± 16.6% nitrogen free extract. It is obvious that CW is relatively high in crude fiber and nitrogen free extract and contain CP levels similar to those found in corn and alfalfa hay but much lower than those found in soybean meal.

Research designed to utilize CW as a feedstuff can be divided into three major areas; utilization of (1) unfermented; (2) aerobically fermented; or (3) anaerobically fermented CW.

Unfermented

Incorporation of unfermented CW in ruminant rations has yielded mixed results. Menear and Smith (86) fractionated dairy manure with a continuous-fed screw press into two fractions: a solid fibrous fraction and a liquid fraction which contained 40 - 47% of the TN orginally present in the waste. The liquid fraction, when incorporated at 0,2.8 or 6% of the dry matter content into a pelleted complete diet, was found to neither adversely affect nor stimulate lamb performance, as determined by digestibility and nitrogen balance trials (113).

Anthony (6) incorporated fibrous portion of CW, which had been washed and screened, or raw unfractionated CW at the 40% level in a basal high energy ration composed of 16% soybean meal and 64% ground ear corn. Performance of steers and bulls fed the ration was comparable to animals fed a

Table 2. Proximate analysis of cattle waste

Reference	Type of Waste	Dry Matter	Crude Protein[1]	Ash	Ether Extract	Crude Fiber	Nitrogen Free Extract
		(% Dry Weight Basis)					
Bhattacharya and Taylor (12)[2]	Bovine,steer	100.0	20.3	11.5			
	Bovine, cow	100.0	12.7	16.1	2.5	37.5	29.4
Ichhponani and Lodhi (63)	Bovine, cow	100.0	12.4	20.9	1.1	51.5	14.0
Lucas et al. (81)	Bovine	91.6	13.2	5.4	2.8	31.4	47.2
Thorlacius (86)	Bovine	100.0	14.8	14.0	2.6	30.5	
National Research Council (93)	Alfalfa hay (1 00 078)[3]	100.0	17.0	9.9	1.9	30.6	40.6
	Corn grain (4 02 879)	100.0	10.9	1.6	4.7	2.4	80.3
	Soybean meal (5 04 607)	100.0	50.5	6.5	1.1	6.0	35.9

[1]Crude protein defined as total nitrogen x 6.25.

[2]Figures reported by these authors are averages of values given by other authors.

[3]National Research Council reference numbers are given in parentheses.

control high energy ration.

Dehydration of CW resulted in a stable product, which was lower in CP content as compared to that in fresh material, and appeared to negatively affect the nutritional quality of the waste. Lucas et al. (81) substituted 20% dried CW in a basal corn-hay-soybean ration. Depressions ($P < .01$) in apparent digestibility of organic matter, CP, crude fiber, ether extract, nitrogen-free extract, acid detergent fiber and energy occured with the CW substituted ration compared with the control ration not containing CW. Thorlacius (122) and others (95) also found that although ruminants would consume large amounts of dehydrated CW, the low digestibility of the latter limited its value as a ration component.

Aerobic Fermentation

Cattle waste is potentially valuable as a substrate for aerobic biomass production (see review, ref. 104). Microbial conversion of feedlot waste fiber into single cell protein (SCP), using thermophilic actinomycetes, was reported by Bellamy (9). Fiber from CW was pretreated with butanol/water (1:1) at 180 C for 30 minutes and fermented. Optimum conditions for cellulase formation and SCP production were reported to be 55 C and pH 7.5 - 7.8, however, actual SCP yield was not reported.

Griffin et al. (47) reported that fresh feedlot waste (FLW) is an excellent substrate for the production of cellulolytic enzymes by *Trichoderma viride*. The fermented

waste was odor free, contained all of the original nitrogen, had 24% less organic matter and the mycelial product was suggested as a potential protein supplement but the protein content in the product was too low to be practical as a feed supplement. Cellulase activity, however, was inhibited at FLW concentrations greater than 2.5%. In contrast, Kaneshiro et al. (66) used feedlot waste fiber and found no cellulase inhibition at substrate concentrations up to 16.7%, although nutrient deficiencies were noted and the fermentation required 10 - 13 days for completion.

Morrison et al. (91) described procedures for the extraction and recovery of the protein constituents of FLW and subsequent fermentation of the extracted residues for the production of additional biomass. Cellulase and SCP were produced in dual culture fermentation of FLW fiber. Cellulase from *T. viride QM9414* was partially purified and used for hydrolysis of diluted FLW (5%) for 18 h at 50 C. Following hydrolysis, FLW was inoculated with *Candida utilis* and incubated for 24 h. Biomass production was 0.71 g/L. However, the practical feasibility of this process was questioned by the authors.

Sloneker et al. (111) fractionated CW into two fractions: a solid fibrous fraction and a liquid portion which contained approximately 72% of the TN originally in the waste. Approximately 45% of the total CP in the liquid was shown to be of microbial origin (91). The above fractionation, followed by separate processing of the two fractions, was

considered essential for the fullest utilization of the waste (103). Weiner and Rhodes (130,131) reported on controlled aerobic fermentation of feedlot waste filtrate utilizing either natural flora or selected fungi and streptomycetes. These studies were primarily concerned with the reduction of biological oxygen demand, chemical oxygen demand, and odor problems associated with FLW and secondarily with the production of microbial protein as a byproduct. Weiner and Rhodes (131) further reported that addition of a readily fermentable carbohydrate source to CW liquid significantly increased biomass yield of streptomycetes and various fungi sixfold. Rhodes and Orton (103) developed a solid-substrate fermentation system in which CW liquid was added to cracked corn at a ratio of 1:2 and the mixture (40% moisture content) was fermented aerobically at 35 - 37 C in a revolving mixer. Fermented corn had a CP content of 10.8% as compared to 9.6% CP in unfermented corn, or a marginal 12.5% improvement in CP due to fermentation.

Anaerobic Fermentation

Anaerobic processes are more economical than aerobic processes because the latter require high capital investment and very substantial aeration costs. Secondly, much of the substrate carbon is lost as carbon dioxide in aerobic processes but in anaerobic processes most of the substrate carbon is conserved in the form of various fermentation products, primarily organic acids and alcohols (59).

However, unlike aerobic fermentations, microbial cell yield is relatively low in anaerobic fermentations.

Partial anaerobic fermentation of harvested forages by the ensiling process is a commonly employed technique in ruminant feed storage. Ensiling CW has been investigated as a waste recycling method (6). Daily gain and intake was superior in lambs fed corn forage ensiled with the liquid fraction expressed from dairy manure, as compared to similarly ensiled corn forage treated with urea or soybean meal (46). Heifers fed wastelage (obtained by mixing and ensiling 57% CW : 43% hay) supplemented with 227 g of a protein-mineral-vitamin A mixture, performed similar to animals receiving a comparable ration without wastelage. Other heifers fed 40% wastelage combined with 60% concentrates performed as well as those fed controlled rations containing 79% corn and 20% grass pellets (95). Harpster et al. (56) reported that growth rate and carcass quality of steers fed up to 40% wastelage and 60% high moisture corn were similar to controls not receiving wastelage. However, wastelage was found to be of limited value when fed alone.

Knight et al. (67) studied the microbial flora of ensiled bovine manure blended rations which are similar to wastelage. Total bacterial counts, number of acid-producing bacteria and numbers of yeasts and molds decreased after 10 days of ensiling. The predominant microorganism at '0' time was *Streptococcus faecalis* whereas after ensiling for 10 days *Lactobacillus plantarum* was the most commonly

isolated organism. Salmonellae were not recovered from salmonella-positive, manure-blended rations after 3 days of ensiling. Coliforms were also not detected in ensilage containing 40 or 60% manure after 5 days of fermentation. However, 10 days were required to decrease coliform populations to undetectable levels in ensilage containing only 20% manure. These results indicated that ensiling CW is a potential means of recycling the nutrients present in the waste as feed; however, ensiling is limited to crop harvesting periods and has the additional disadvantage of long fermentation time.

Other processes have been investigated as a means of recycling agricultural waste on a more regular and efficient basis. Moore and Anthony (90) fermented CW at 37 C for 72 h under anaerobic conditions. The pH of the fermentation was adjusted to 6.25 every 24 h by the addition of ammonia. As a result of fermentation and pH control the CP content in the product was 43.3% as compared to 17% in the starting material. In palatability tests with lambs, the fermented CW was equal to control rations containing no manure, however, further details are not available.

Organic acids were shown to be valuable energy sources for ruminants (62). Supplementation of low, basal or high protein diets with 5% organic acids has been shown to result in a 9% improvement in gain in steers compared to similar diets without supplementation (60). Furthermore, ammonium salts of organic acids were shown to be excellent nonprotein nitrogen sources for ruminants (31). Allen and Henderson (2)

showed that cattle receiving ammonium salts of organic acids as their CP supplement had average daily gains comparable to steers fed soybean meal and superior to steers fed urea supplemented rations. Milk production of cows was similar when ammonium lactate, urea or soybean meal furnished 27% of the TN in the rations containing 13% CP (61). Chemically pure ammonium salts of organic acids or fermented ammoniated condensed whey in which ammonium lactate comprises 75% of the CP have also been shown to be less toxic than urea when fed to ruminants (28, 102). It would appear, therefore, that anaerobic fermentation of livestock wastes to produce organic acids, and utilization of the ammoniated form of the organic acids as a ruminant protein-energy supplement is potentially advantageous from a nutritional viewpoint.

The recycling of agricultural wastes as nitrogenous feed supplements for ruminants via ammoniated organic acid fermentations has recently been reviewed by Gerhardt and Reddy (43). In these processes organic acids are produced by microbial fermentation of carbohydrate rich agricultural wastes under conditions of controlled pH and temperature. Ammonia is automatically added to the fermentor to maintain a constant pH by partially neutralizing the organic acids produced and also to increase the CP content in the fermentation product. The product is stabilized by evaporation or other means and fed as a protein feed supplement to ruminants. The feasibility of this approach has already been documented with whey, a watery byproduct of cheese manufacturing (102)

and other agrio-industrial wastes (41). Also, the ammoniated organic acid fermentation process is superior to aerobic SCP production in terms of CP content because of the additional increase in CP due to the ammonia nitrogen.

Recycling Poultry Waste as a Feedstuff

Poultry waste (PW) contain on the average (Table 3) 32.2 ± 6.9% CP, 20.1 ± 6.7% ash, 3.0 ± 2.0% ether extract, 14.0 ± 3.3% crude fiber and 30.8 ± 4.8% nitrogen free extract. It is obvious that PW contains more CP on a dry basis than alfalfa hay (see Table 2) and corn grain and has 64% as much CP as soybean meal. However, ash content is considerable higher and net energy values lower in PW as compared to the latter three ration components.

Recycling PW as a feedstuff can be divided into four major research areas: (1) unfermented; (2) aerobically fermented; (3) anaerobically fermented and (4) insect biomass production.

Unfermented

As indicated above PW is rich in CP, most of which is in the form of non-protein nitrogen (NPN), primarily urea and uric acid. Since the ability of ruminants to utilize NPN supplements for satisfying their dietary nitrogen requirements, raw PW has been directly incorporated into ruminant rations at levels of 0 - 65% (33,34,38,68,85,117). Weight gains and feed/gain ratios were not significantly ($P > 0.05$) different when PW was incorporated into rations at the 10%

Table 3. Proximate analysis of poultry waste

Reference	Type of Waste	Dry Matter	Crude Protein	Ash	Ether Extract	Crude Fiber	Nitrogen-Free Extract
		(% Dry Weight Basis)					
Bhattacharya and Fontenot (11)	Broilers	89.1	32.0	17.9	2.8	15.1	31.8
	Broilers	88.9	30.6	19.0	2.8	14.6	33.1
Bhattacharya and Taylor (12)[1]	Layer	89.7	28.0	28.0	2.0	12.7	28.7
Biely et al. (13)[2]	Layer	90.4	25.6	20.9	2.0	15.8	
Biely and Stapleton (14)[2]	Chick	93.1	28.0		2.6	9.3	
Blair and Knight (17)[1]	Layer	88.6	28.7	26.5	1.8	13.8	35.6
	Layer	84.5	25.3	14.1	2.3	18.7	27.1
Chang et al. (24)[2]	Layer	100.0	30.3		1.7	11.0	
	Layer	100.0	29.5		1.4	10.9	
Cullison et al. (30)	Layer	93.2	40.4	27.0	0.5	8.9	23.1
	Broiler	88.3	34.5	19.1	2.2	11.4	32.8
Goering and Smith (46)	Layer	87.8	28.8				
Harmon et al. (53)	Broiler	84.3	38.9	11.7	5.6	17.5	26.3
	Broiler	87.4	34.7	11.6	4.5	16.8	32.3
	Broiler	99.7	43.1	11.2	5.8	18.5	30.4
	Broiler	99.1	37.2	12.2	5.5	17.2	27.9
Ichhponani and Lodhi (63)[1]	Poultry	100.0	30.0	20.7	1.9		
Oltjen and Dinius (97)[2]	Layer	96.3	31.3	31.3			
Silva et al. (109)	Layer	76.1	15.3	13.5	1.2	16.5	29.5
Stapleton and Biely (114)[2]	Cockerels	100.0	25.7		3.1	11.7	
	Cockerels	100.0	29.7		9.6	10.5	
Swingle et al. (115)	Layer	94.0	42.3	29.0	2.3		
Teotia and Miller (120)	Layer	100.0	46.9	22.7	2.6	18.1	42.1
Zindel et al. (137)	Layer	100.0	35.6	25.7	2.0	11.1	

[1]Figures reported by these authors are averages of values given by other authors.

[2]Average of values given by authors.

level when compared to conventional, PW-free diets (68); however, amounts of PW in the rations greater than 10% decreased CP and energy digestibilities.

Extensive research efforts have been directed at recycling dried poultry waste (DPW) as a feed for chickens, ruminants and swine. DPW contains greater than 85% dry matter and a CP content of approximately 33% (27). Extensive studies with DPW at Michigan State University (106,107,108,137) showed that it is chemically stable for 1.0 - 1.5 years (24,48) and contains 5.9×10^3 - 3.6×10^6 microorganisms/g. The most frequent isolates were *Bacillus* and *Streptococcus*. *Salmonella*, *Proteus*, *Neisseria*, *Penicillium* and other molds were not detected (23,48).

Various animal types respond differently when fed DPW. The nutritional value of DPW when substituted for a portion of a standard energy-protein-mineral ration for chicks (13, 14,72,110,114,124,125), broilers (13,30,73,124) and hens (13,15,74,75,84,124) has been thoroughly investigated. Growth rate and feed efficiency were not affected when the rations contained 0 - 10% DPW. However, growth rate and feed efficiency progressively decreased as the level of DPW incorporated into the rations increased from 10 - 30% when compared to DPW-free rations. The nutritional value of DPW when fed to ruminants has also been investigated (11,20,29,32,37,45, 53,80,96,97,109,115,121,123). Weight gain and feed intake of ruminants fed typical corn-soybean meal rations substituted with up to 20% DPW were similar to control animals fed

DPW-free rations. Decreases in weight gain and feed intake occured, however, only when DPW concentrations exceeded 20% of the ration (29,39,109). Aleman et al. (1) fed growing swine conventional rations substituted with DPW at the levels of 0,10,20, and 30%. All swine fed DPW-substituted rations remained healthy and the DPW had no apparent adverse effect on the carcasses. However, for every 10% increase in DPW substituted in the rations growth rate was reduced by 0.02 kg/d, feed conversion efficiency decreased by 0.25 units and killing-out percentage was reduced by 0.96 which was significantly ($P < 0.01$) different as compared to swine fed the DPW-free diet. These results indicate that production of DPW is a viable waste handling system which produces a stable product that is also nutritionally valuable when substituted in conventional rations at levels up to 10 and 20% in chicken and ruminant diets, respectively. However, production of DPW entails high energy requirements for the drying process and the loss of valuable nutrients due to volatilization and thermal treatment. These losses must be minimized to increase the feasibility of recycling PW by this method (27, 33, 34, 38, 68, 82, 105).

Aerobic Fermentation

Jackson et al. (64) investigated the feasibility of increasing the microbial biomass of PW by aerobic fermentation. Fresh PW was diluted 1:10 (w/v) and blended with sterile 0.1% peptone-water. The slurry obtained was transferred to flasks which were sealed, gassed continuously

with sterile air, agitated at 120 rpm and incubated at 37 C for 7 d. Samples incubated under these conditions showed an increase in total bacterial counts beginning after 24 h and continuing until the end of fermentation (7 d). The increase represented a change in total counts from 8.0×10^7 - 1.6×10^9 bacteria/ml of culture. Before this process can become economically competitive with alternative waste management systems, more research must be conducted to reduce the long fermentation time (4 - 7 d) and costs of aeration while substantially increasing cell yields.

Vuori and Nasi (127) isolated microbial strains which assimilate uric acid and selected a yeast strain which optimally utilized uric acid for the fermentation of PW in an attempt to increase its nutritional value for poultry diets. After supplementation of PW with glycerol sterilized laboratory scale (10 - L) batch fermentations and non-sterile semi-continuous pilot scale (150 - L) fermentations were conducted. The uric acid in PW and added glycerol were metabolized within 1 and 3 d, respectively, and the total amino acids (tryptophan content not included) concentration increased from 76 - 136 g/kg during fermentation. Further nutritional evaluation of the fermented product was not reported.

Anaerobic Fermentation

Fermentation of PW by ensilement has been reviewed by Syrett (116). Caswell et al. (21) ensiled PW (containing 15.6% moisture) alone and with sufficient distilled water

added and found optimum fermentation (as determined by pH and acid concentrations) to occur at a 40% moisture level. PW has also been ensiled with ground corn (22), corn forage (54,55), tree bark (71) and in a ration treated with tannic acid or paraformaldehyde (38). The coliform populations in ensiled PW or PW mixtures were generally lower than controls (21,71). Silage characteristics were not adversely affected by PW (38). Feeding trials with sheep indicated that litter nitrogen was well utilized when incorporated into rations up to the 15% level. Further increasing the level of litter nitrogen from 15 to 30% of the silage dry matter depressed efficiency of dietary nitrogen utilization 13% (55). Other reseachers (21,22) found similar depressions in nitrogen retention when ensiled PW nitrogen represented 50% of the ration nitrogen; however, digestion coefficients were not significantly different. In contrast, Jacobs and Leibholz (65) reported the percentage of apparent digestion occurring in the stomach to be higher when the diets contained PW ensiled with sorghum (2:1) and incorporated into the ration at the 45% level; however, significantly ($P < 0.05$) less nitrogen was digested in the hind gut with the same ensiled PW ration when compared to rations containing 30 and 50% raw PW or control rations.

Jackson et al. (64) investigated the feasibility of increasing biomass in PW through anaerobic fermentation. The PW was treated as described above for the aerobic fermentation of PW; however, sterile nitrogen was bubbled

into the flasks. Samples collected throughout the fermentation showed little variation in total bacterial counts during the first 3 days of incubation. After 3 days, the number of bacteria decreased sharply and this trend continued throughout the remainder of the fermentation. The decrease represented a change in total bacterial counts from 1.1×10^8 - 7.0×10^5 bacteria/ml of culture. No attempt was made by these investigators to identify changes in the PW slurry that may have been brought about by the anaerobic microorganisms.

Insect Biomass Production

The production of fly (Diptera) biomass from PW has been reported by Miller et al. (87) and Teotia and Miller (119, 120). Fly pupae (*Musca domestica*) were produced in PW under controlled temperature and moisture conditions. A yield of 19.0 g of dry pupae/kg of fresh PW were obtained in 8 d and there was a 48% reduction in weight of the PW on a dry basis; the organic matter content was reduced by 80%. Chemical alalysis showed that pupae contained 61.4% CP, 9.3% fat and had an amino acid profile comparable to meat and superior to soybean meal. Nutritional trials with chicks fed a complete ration containing 30% pupae showed no difference in weight gain or feed conversion efficiency as compared to control chicks fed soybean meal. In contrast, feed conversion in chicks fed 30% digested residue (the remains after harvesting fly pupae) was significantly ($P < 0.05$) inferior to PW-free control rations. The results of feeding digested PW were

similar to results obtained when DPW was fed to chicks at the 30% level (13). Newton et al. (94) also investigated the nutritional value of larvae (*Hermetia illucens*) collected from CW as a supplement for monogastric animals. Consumption of a diet containing 33% larvae by pigs was significantly ($P < 0.05$) greater than that of a control soybean meal diet. In contrast, apparent digestibilities of dry matter, nitrogen, ash and nitrogen-free extract were significantly ($P < 0.05$) greater for the soybean control diet as compared to the larvae diet. The data to date suggest that production of insect biomass from PW is not a practical proposition for recycling PW as a feedstuff because of low yields of pupae, long generation time and the low nitritional value of the digested residue.

Recycling Swine Waste as a Feedstuff

Previous reports indicate (Table 4) that swine waste (SW) contain on the average 17.2 $\pm$ 5.6% CP, 17.2 $\pm$ 1.3% ash, 4.9 $\pm$ 2.4% ether extract and 43% nitrogen-free extract. CP content of SW is higher than that in corn, equivalent to that in alfalfa hay, and 34% of that in soybean meal (see Table 2). Thus, SW appears to have a promising nutritional potential as a feedstuff. However, little work has been done on the recycling of unfermented or fermented SW as a feedstuff.

Unfermented

Orr (98,100) substituted dried SW into swine finishing diets. Feed consumption, daily gain and feed efficiency were

Table 4. Proximate analysis of swine waste

Reference	Dry Matter	Crude Protein	Ash	Ether Extract	Nitrogen-Free Extract
	(% Dry Weight Basis)				
Blair and Knight (16)[1]	10.0	10.9	16.2	3.2	43.0
Orr (98)	100.0	21.7			
Pearce (101)[2]	100.0	18.9	18.1	6.6	

[1] Figures reported by these authors are averages of values given by other authors.

[2] Average of values given by author.

reduced 7,39 and 55%, respectively, when the swine were fed a diet containing 78% corn and 22% dried SW as compared to animals receiving rations containing 86% corn and 12% soybean meal. Feed consumption was reduced 5% when swine were fed a ration containing 20% dried SW, 58% corn, 10% soybean beal, 4% corn oil and 5% cane sugar when compared to similar animals fed a 86% corn-12% soybean meal ration (99). Kornegay et al. (70) substituted dried SW and unprocessed fresh SW at levels of 21.7 and 37.3%, respectively, in a basal corn-soybean meal ration and found the SW supplemented rations to be of lower nutritive value than a control ration without SW.

Miller (88) fed growing-finishing swine a corn-soybean meal diet and provided waste water from a swine waste flushing system as a sole source of drinking water. Feed intake and weight gains of pigs receiving this treatment, particularly those under 45 kg, were depressed when compared to similarly fed pigs receiving fresh drinking water. Finishing pigs, however, adapted to this treatment and performed well after an initial depression period. Although, this feeding regime would reduce ration mineral requirements, the level of protein in the ration could not be reduced since most of the nitrogen in the waste water was in the form of NPN.

The value of SW as a nitrogen and energy source for ruminants has been investigated but with disappointing results. Pearce (101) fed steers diets in which dried SW

replaced hay at levels of 0,15,30, and 45% in a basal hay (93%) - molasses (3%) diet. Dry matter digestibility decreased linearly from 58 - 44% for diets containing 0 - 45% dried SW. Tinnimitt et al. (123) also reported low digestibilities comparable to those of Pearce (101), for sheep fed a corn-corn cob diet containing 32.4% dried SW. The above data indicate that SW has little practical value as a feedstuff for steers.

Fermented

Orr (99) evaluated SW oxidation ditch liquor (ODL) as a source of nutrients in finishing diets for swine. Average daily feed intake and daily gains were depressed 7 and 14%, respectively, in swine on corn-limited soybean meal-ODL rations as compared to swine on standard corn-soybean meal-fresh water diets. In contrast, dried ODL substituted for corn at levels of 0,10,20 and 30% in a basal corn-soybean meal diet significantly ($P < 0.05$) improved weight gains and feed efficiency of finishing swine which were fed a diet marginal in protein (51,52).

Henry et al. (58) grew a pellicle forming yeast, *Candida ingens*, on effluent taken from a commerical swine farm. Under laboratory conditions *C. ingens* utilized 38% (by weight) of the fermentation acids and 20% (by weight) of the ammonia nitrogen, in anaerobically fermented SW for growth. Yield of SCP (dry basis), after 39 h of growth, was 1.39 g/L. This yeast product contained 44.5% CP, 21.7%

nitrogen-free extract, 21% ash, 2.3% ether extract and 0.3% fiber, and is a potentially valuable protein, mineral and B-vitamin supplement in feeds (57). However, the overall feasibility of implementing such a process on SW management may be minimal in view of the low yield, time duration, expense involved in product recovery and the disposal of the effluent remaining after cell harvesting.

Laboratory and pilot scale aerobic, solid substrate fermentation of corn supplemented with SW (2:1, w/v) was investigated by Weiner (128,129). The mixture was agitated (0.5 rpm) and incubated at 18 - 28 C for 1.5 - 6.0 d. The initial microflora present in SW was heterogenous; however, within 48 - 72 h, lactobacilli and yeasts were predominant (92). Growing pigs fed 80% fermentation product and 16% soybean meal accepted the ration but gain and gain/feed were reduced 33% when compared to similar animals fed 78% corn and 19% soybean meal.

The symbiotic growth of bacteria and algae in SW as a means of reducing biological oxygen demand (BOD) and chemical oxygen demand of SW has been investigated by Garrett and Allen (42) and Wilson and Houghton (132,133). Growth of indigenous bacteria and algae resulted in a 61% decrease in permanganate value, 16% decrease in BOD and a 23% decrease in ammonia concentration while potentially providing an algae crop as a byproduct for use as an animal feed supplement. Further research is needed to optimize production and evaluate the nutritional value

of the biomass.

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SECTION 1 (ARTICLE 1)

PRODUCTION OF A RUMINANT PROTEIN SUPPLEMENT BY ANAEROBIC FERMENTATION OF FEEDLOT WASTE FILTRATE

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Production of a Ruminant Protein Supplement by Anaerobic Fermentation of Feedlot Waste Filtrate*

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INTRODUCTION

Nearly all plant proteins presently fed to feedlot cattle can be consumed by man. Yet, feedlot cattle convert only about 15% of the plant protein consumed into red meat. It is estimated that feedlot steers need nearly 18 lb of grain to produce 1 lb of red meat. It is hard to justify such an inefficient use of our protein resources at a time when there is a world-wide shortage of protein. Furthermore, because of world affluence, population increases, and the presence of political barriers that restrict international trade, there has been a substantial rise in the demand for plant proteins. As a consequence, the prices of plant proteins have gone up and a situation has arisen wherein animals have to compete with man for their protein requirements. This is an unhealthy trend and has contributed to significant raises in the price of meat. Consequently, there is an obvious need for developing inexpensive alternative sources of protein for feeding cattle. Preferably, these alternate sources of protein should be derived from materials that are not a part of the human food chain.

For the past three years, we at Michigan State University have been involved in the development of inexpensive sources of crude protein feed supplements by recycling agricultural waste materials. We are interested in agricultural wastes for the following reasons:

1) Agricultural wastes are produced in large quantities. For example, nearly two billion tons of animal wastes alone are produced annually in the United States.

2) Most of these wastes have a high biological oxygen demand (BOD) and, as such, are a potential polution hazard. It is very expensive to dispose of these wastes within federal environmental standards.

3) Wastes contain large amounts of potentially usable proteins and carbohydrates. In view of the increasing world-wide demand for protein now, and the expected doubling in demand for protein in the next 30 years, it would be desirable to recover the proteins and carbohydrate materials present in the wastes.

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4) The caloric value of wastes is recoverable as biogas and/or ethanol.

5) Most of the agricultural wastes are renewable and, hence, there is no shortage of fermentable substrate.

6) Often, there is little cost involved in recovering the wastes.

7) In nearly all cases, agricultural wastes are not a part of the human food chain, and any process for recycling the wastes will not compete with man's food sources.

As Taiganides [1] pointed out, wastes are really "resources out of place or out of time" and it is very important that maximum efforts be made to turn our "effluents into affluence."

We chose to investigate the feasibility of recycling feedlot waste (FLW) as a feed supplement for ruminant animals for rather obvious reasons. FLW is produced in a large concentrated volume in confined areas. It is estimated that out of more than two billion tons of farm animal wastes produced annually in the United States, nearly half such wastes are from intensive animal production systems such as feedlot operations. The traditional practice of disposal of animal manure onto land is not practical because of the limited land available for the disposal of large tonnages of waste, and the high disposal costs. FLW represents a growing and critical problem with respect to environmental pollution because FLW has a relatively high BOD and chemical oxygen demand (COD) which readily support the growth of a number of microorganisms, which when allowed to ferment uncontrolled, will result in the production of objectionable odors, attract insects, or when released into water, quickly deplete dissolved oxygen and kill aquatic zooplankton and phytoplankton [2]. Also, animal wastes are rich in nutrients, a large part of which are recyclable. Taiganides and Stroshine [3] indicated that the beef cattle manure produced in one day in the United States contains nearly 20×10^6 kg of total nitrogen or 125×10^6 kg of crude protein (total N $\times$ 6.25). This is several times greater than the crude protein present in all the soybeans that are presently fed to cattle in the United States.

The recycling of animal waste as a feed ingredient for livestock was reviewed by Anthony [4, 5], Smith [6, 7], and most recently by Bhattacharya and Taylor [8]. Sloneker et al. [9] fractionated waste into solid and liquid portions and suggested the possible use of the latter fraction as a protein feed supplement. A number of studies have been done on the controlled aerobic fermentation of either feedlot waste liquid utilizing natural flora or unfractionated FLW utilizing *Trichoderma viride* [9–11]. These studies were mainly concerned with reducing the BOD, COD, and odor problems associated with FLW and to produce microbial protein as a by-product. Coe and Turk [12] investigated the economic feasibility for producing methane by anaerobic fermentation of FLW and suggested the possible use of the fermentor effluents as a cattle feed. The present study was initiated to develop simple and efficient procedures for the production of feed supplement(s) rich in crude protein by anaerobic fermentation of the liquid portion of the FLW.

EXPERIMENTAL

Fractionation of FLW

FLW including urine, straw, and wasted feed particles (one day old) was collected from concrete floors of a small feedlot operation wherein the steers were fed a ration of 60% high moisture shell corn and 40% corn silage. FLW was diluted either 1:9 (w/w) or 1:1 (w/w) with tap water for different experiments and mixed thoroughly to obtain an even slurry. This FLW slurry was then filter pressed through a two-layered cheesecloth. The filtrate containing most of the solubles and some fine particulate matter of the original FLW is referred to as feedlot waste filtrate (FLWF) throughout the rest of this paper. FLWF was used for fermentations within 4 hr from the time of collection. The solid portion retained on the cheesecloth is referred to as the fiber portion. The composition of the fiber fraction and FLWF presented in Table I, is similar to that of Sloneker et al. [9]. The fiber fraction is rich in cellulose and noncellulose carbohydrate (mainly hemicellulose) and low in nitrogen and ash. FLWF fraction, on the other hand, is low in cellulose and hemicellulose, high in ash and total nitrogen (about 60–70% of the total nitrogen in FLW), and accounts for up to 20% of the total solids present in FLW.

Carbohydrate Sources Used to Supplement FLWF

Waste carbohydrate sources used to supplement FLWF in various fermentations were molasses (Corn Products Corporation International, Arco, Ill.), corn starch (Staley Manufacturing Co., Oakbrook, Ill.), acid whey or whey powder (Michigan Milk Producers Association, Ovid, Mich.), sweet whey (Michigan State University Dairy Plant, E. Lansing, Mich.), and starch recovered from potato processing wastes (Allied Foods, Livonia, Mich.). In some experiments pure dextrose or sucrose (J. T. Baker Chemical Co., Phillipsburg, N.J.) was used as the supplemental carbohydrate.

TABLE I

Composition of Fiber and FLWF Fractions of the FLW

Component	Fiber (% Dry Matter)	Solubles and Fines (FLWF) (% Dry Matter)
Cellulose	24.5	3.0
Non-Cellulose Carbohydrate	25.0	6.0
Lignin	8.5	7.0
Ash	11.5	35.5
Nitrogen	2.0	3.0-5.2

Fermentation Procedures

A 28 liter Microferm (New Brunswick Scientific Co., New Brunswick, N.J.) fermentor was used throughout this study. The fermentor was operated at the 20 liter level. Since FLWF is low in carbohydrate, a waste carbohydrate source was added at a suitable level to most of the fermentations. Agitation was provided at 250 rpm. The temperature was adjusted to and maintained at 40 or 44°C (±0.5°C) for different fermentations. The pH of the fermentor contents was adjusted to a desired level (see below for details) by the addition of ammonia. Fermentation was initiated by adding a suitable amount of inoculum (see below). An automatic pH recording controller (model pH-22, New Brunswick Scientific) was used to continually record and control the pH of the fermentor contents. The pH in the fermentor was maintained constant (±0.1 pH units) at a preset level as follows: As the pH drops during the fermentation, owing to the production of organic acids, the pH recording controller responds by transmitting a continuous electrical impulse to an actuating solenoid valve (Model X826B220, Automatic Switch Co., Forham Park, N.J.) which then opens and allows the ammonia to be added to the fermentor from a pressurized ammonia cylinder. Ammonia partially neutralizes the organic acids produced and the pH increases. Once the pH rises to the preset level, the pH recording controller terminates the electrical transmission to the solenoid valve and the ammonia addition is terminated. At the end of fermentation (24 hr) the product obtained was concentrated fivefold using a Buchler flash evaporator (Model PF, A. H. Thomas Co., Philadelphia, Penn.). The pH of the condensed product was raised to 6.8 by adding additional ammonia. This last step further elevates the crude protein level in the product. The final fermented, condensed, ammoniated product is referred to as "FEED PRO" and contains ammonium salts of organic acids and single cell protein as the main nitrogenous components.

In fermentations utilizing mixed rumen bacteria as the inoculum, FLWF with or without added carbohydrate was sterilized under O_2-free CO_2 gas, and sterile, anaerobically prepared cysteine hydrochloride reducing solution was added to give a final concentration of 0.1% (w/v). Temperature and pH were adjusted to 40 and 6.5°C, respectively, and were maintained at this level throughout the fermentation. The rumen contents freshly collected from a fistulated Holstein cow, maintained on a hay-silage ration, were aseptically filter pressed through a two-layered cheesecloth into a 3 liter Erlenmeyer flask. The flask was immediately transported to the laboratory (20 min) where the top 1 liter of filtered rumen fluid was poured off and the remainder used to inoculate the fermentor, aseptically and anaerobically, to give a final concentration of 10% (v/v). Anaerobiosis was maintained throughout the fermentation by continuously flushing with O_2-free CO_2 gas.

In fermentations receiving *Lactobacilli* inoculum, no anaerobic or aseptic precautions were taken and no cysteine hydrochloride reducing solution was used. *Lactobacilli* stocks were maintained on slants of trypticase soy agar

(BBL) and stored at 4°C. These cultures were transferred once every month to fresh medium. A loopful of 24 hr slant culture was transferred to 10 ml sterile trypticase soy broth (BBL) in 18 × 150 mm² screw cap tubes and incubated at 44°C for 24 hr. This broth culture served as the inoculum for inoculating 1 liter of sterile trypticase soy broth contained in a 2 liter foam-plugged Erlenmeyer flask. After incubation for 24 hr at 44°C, this inoculum was added to the unsterilized FLWF in the fermentor to give a final concentration of 5% (v/v). The fermentor was then closed to the outside environment. Temperature was maintained at 44°C and the pH at 5.5.

In fermentations receiving no exogenous inoculum, the indigenous flora were responsible for the fermentation. No special sterile or anaerobic precautions were taken except that the fermentor was closed to the outside environment. Temperature was maintained at 44°C and the pH at 5.5.

Fractionation and Fermentation of Manure

The fractionation and fermentation of fresh manure were carried out the same way as for FLWF except fresh manure (not including straw, urine, or feed particles) was substituted in place of FLW.

Analytical

Total nitrogen was determined by the Micro-Kjeldahl technique [13]. Total solids were determined by drying a 50 g sample in a forced air oven at 60°C for 48 hr. The supernatant obtained after centrifugation of the sample at 25,000 *g* for 15 min was used for the determination of ammonia nitrogen [14], total carbohydrate [15], and quantitative analysis of fermentation acids produced [16].

RESULTS AND DISCUSSION

FLW Fermentations Utilizing Mixed Rumen Bacteria

The composition of representative unfermented and fermented FLWF is shown in Table II. The data show that FLWF is low in carbohydrate and the crude protein content of five-times-concentrated FLWF is also low. Anaerobic fermentation of FLWF by rumen bacteria results in utilization of most of the carbohydrate present and a doubling in the crude protein content of the product. However, it is not practical to use either the product obtained from unfermented FLWF or that obtained by fermentation of FLWF, not supplemented with exogenous carbohydrate, because both these products are very low in crude protein content and total solids and are highly susceptible to microbial and fungal spoilage.

Anaerobic fermentation of FLWF supplemented with cheese whey to give a final concentration of 3.5% lactose or with pure dextrose (3.1%) or sucrose (3.3%) results in a product that has a much higher crude protein content as compared to the product obtained by fermentation of unsupple-

TABLE II

Composition of Unfermented and Fermented FLWF[1,2]

Component	Unfermented FLWF %	Fermented FLWF %	Fermented FLWF + 3.5% Lactose (Whey), %	Fermented FLWF + (3.1% Dextrose) %	Fermented FLWF + (3.1% Sucrose) %
Initial Carbohydrate	0.50	0.50	3.50	-	-
Final Carbohydrate		0.03	0.19	-	-
[3] NH_3-N	0.05	0.25	2.47	1.45	1.81
[3] Total N	0.30	0.65	2.87	2.10	2.43
[3] Crude Protein (Total N x 6.25)	1.87	4.06	18.00	13.12	15.18
[3] Total Solids	3.75	3.10	21.00	20.50	20.00

[1] FLW which is diluted 1:9 with water and filter-pressed through a two-layered cheese cloth is referred to as FLWF.

[2] Fermentations were run anaerobically, under CO_2 gas phase, at a temperature of 40°C and pH 6.5.

[3] These figures represent values for a five times concentrated product.

mented FLWF. The product has a crude protein content of 13–18% on a 20–21% solids basis or 65–85% crude protein on a dry basis. In a number of similar experiments we consistently get a product that has a crude protein content of 70–80% or higher on a dry (100% solids) basis. The efficiency of utilization of added carbohydrate is 90% or higher, and the time required for fermentation is 24 hr or less. Ammonia nitrogen constitutes about 70–80% of the total nitrogen in the product. Most of the ammonia nitrogen is present as ammonium salts of acetic, propionic, and butyric acids which constitute 90% or more of the total organic acids in the product. Valerate, lactate, succinate, isobutyrate, and isovalerate are present in trace amounts. The acetate:propionate:butyrate ratio in whey plus FLWF product was 50:44:4, respectively. Similar determinations have not been done with the product from FLWF plus sucrose, or FLWF plus dextrose fermentations. Ammonium salts of organic acids have been shown to be comparable to soybean meal as crude protein feed supplements to ruminant animals [17]. The product has little bad odor associated with FLW. The product is brown in color, stable, and resistant to microbial spoilage at room temperature. No microbial spoilage was detectable in the product even after storing it for six months at room temperature.

Fermentation of FLWF by *Lactobacilli*

Strict anaerobic fermentations are difficult, cumbersome, and expensive. Therefore, the feasibility of fermenting carbohydrate supplemented FLWF with *Lactobacilli* was investigated. There are several advantages in using *Lactobacilli*: 1) they are aerotolerant anaerobes and do not require strict anaerobic conditions; 2) they efficiently ferment carbohydrates to lactic acid; 3) they grow well at a relatively high temperature of 44°C and low pH of 5.5, which is detrimental to the growth of most contaminant microorganisms, and 4) they tolerate high lactic acid concentrations inhibitory to many microorganisms.

The results presented in Table III show that the crude protein content of the product (29–35%) is significantly higher than that produced in strict anaerobic fermentations using rumen fluid inoculum. In several fermentations, supplementation of FLWF with either lactose or molasses results in products that are similar in crude protein content. However, the molasses product has lower crude protein based on total solids content because half of the total solids in molasses represents noncarbohydrate material which is nonfermentable. Ammonia nitrogen accounts for 70 to 80% of the total nitrogen in the final product and in this and in most other respects the product produced is similar to that obtained in fermentations receiving rumen fluid inoculum. Fermentations are essentially complete in 24 hr and sugar concentrations up to 5% can be metabolized with 95% or greater efficiency during the fermentation. The results also indicate that FLWF obtained from 1:1 dilution of FLW (with a 2–3% total solids) can be fermented as efficiently as FLWF obtained from 1:9 dilution of FLW (with a 0.7–0.9% total solids level). Utilization of FLWF obtained from 1:1 dilution of FLW results in a greater amount of recycled FLW solids per unit of fermentor volume, which results in lower capital investment and processing costs. Therefore, in all subsequent fermentations a 1:1 FLWF was used.

Fermentation of FLWF by Natural Flora

We then evaluated the ability of native flora to successfully ferment FLWF (supplemented with a carbohydrate source) without any inoculum added, without providing any special anaerobic conditions, and without sterilizing the substrate. The results of several FLWF fermentations supplemented with whey, molasses, potato starch, or corn starch and fermented with the indigenous flora are presented in Table IV. The results show that natural flora present in FLWF efficiently ferment the added carbohydrates

TABLE III

Fermentation of FLWF[1] Supplemented with Carbohydrate by *Lactobacilli*[2]

Treatment[3]	Inoculum	Initial Material		Final Material		Concentrated 5X	
		Total N %	NH_3-N %	Total N %	NH_3-N %	CPE %	Total Solids %
FLWF + Lactose (Whey)	L. bulgaricus	0.27	0.06	5.65	3.95	35.3	24.4
FLWF + Sucrose (Molasses)	L. bulgaricus	0.20	0.06	4.65	3.35	29.1	42.7
FLWF[4] + Sucrose (Molasses)	L. thermophilus + L. delbrueckii	0.30	0.01	4.65	3.80	29.1	42.4

[1] FLW diluted 1:9 (w/w) with water and filter-pressed through a two-layer cheesecloth.

[2] Fermentations were run at 44°C and pH 5.5 for 24 hr under nonsterile conditions.

[3] Carbohydrate sources were added to give an initial concentration of 5% (w/v).

[4] Refers to FLW diluted 1:1 (w/w) with water and filter pressed through a two-layered cheesecloth.

[5] CPE refers to crude protein equivalent (total nitrogen times 6.25).

TABLE IV

Fermentation of Carbohydrate-Supplemented FLWF[1] by Natural Flora[2]

Carbohydrate Source[3]	Initial Material			Fermented, Concentrated 5 X			
	Total N %	NH_3-N %	Total Solids %	Total N %	NH_3-N %	CPE[4] %	Total Solids %
Whey Powder	0.23	0.07	8.0	3.8	2.7	23.7	28.7
Molasses	0.16	0.06	9.9	3.6	2.7	22.5	47.5
Corn Starch	0.18	0.06	7.8	4.0	3.2	25.0	32.0
Potato Starch	0.18	0.05	7.8	3.4	2.5	21.2	28.7

[1] FLW diluted 1:1 (w/w) with water and filter-pressed through two-layered cheesecloth.

[2] Natural flora refers to microorganisms already present in FLWF. Fermentations were at pH 5.5 and 44°C.

[3] Carbohydrate in the initial material was 5.0 ± 0.3% and the residual carbohydrate in the fermented material was 0.3 ± 0.2%.

[4] CPE stands for crude protein equivalent (total nitrogen times 6.25).

and yield a product that is comparable in quality to that obtained from fermentations receiving *Lactobacilli*, as shown in Table III. With the exception of the molasses product, all the others yield a product with a crude protein equivalent of 21–25% on a 29–32% solids basis or 74–82% on a dry basis. Molasses product has a low crude protein based on total solids content because about half of the total solids in this product represents noncarbohydrate material originally present in molasses. If a correction is made for these noncarbohydrate materials, molasses product will have a 80–86% crude protein content on a dry matter basis.

Fermentation of Manure by Natural Flora

The results of experiments designed to evaluate the ability of the natural flora in fresh manure to grow under anaerobic conditions and ferment the added carbohydrate are presented in Table V. Manure was fermented either

TABLE V

Fermentation of Fresh Manure Supplemented with Different Carbohydrates[1]

Treatment	Initial Material		Final Material Concentrated 5 X			
	Total N %	NH_3-N %	Total N %	NH_3-N %	CPE[2] %	Total Solids %
Manure	0.03	0.0	0.20	0.01	1.25	3.75
Manure + Dextrose, 3.5%	0.03	0.004	3.95	3.10	24.68	22.50
Manure + Lactose, 5.2%	0.29	0.026	3.35	2.40	20.93	21.50
Manure + Lactose, 5.0% *L. bulgaricus*	0.20	0.04	3.50	3.15	21.87	20.85

[1] Fermentations were done at a temperature of 44°C, pH 5.5, and under nonsterile conditions for 24 hr. Unless indicated otherwise, no separate inoculum was added. Manure used was diluted 1:9 with paper prior to fermentation.

[2] CPE stands for crude protein equivalent (total nitrogen times 6.25).

by itself or after supplementation with dextrose or lactose (in the form of whey) at the concentrations indicated. Unless otherwise indicated, no separate inoculum was added to these fermentations, other than the indigenous flora already present in the manure. The results show that fermentation of unsupplemented manure results in a product that is very low in crude protein. The natural flora in manure ferment the added carbohydrate efficiently and result in a product that is very similar in crude protein content to that obtained from FLWF fermentations. The results also show that the addition of *Lactobacillus bulgaricus* inoculum to manure plus lactose (5%) fermentation results in a product that is similar in crude protein content to that obtained in manure plus lactose fermentations not receiving *L. bulgaricus*. This indicates that the addition of *L. bulgaricus* has no detectable beneficial effect on these fermentations.

Volatile Fatty Acid Composition

Volatile fatty acid (VFA) composition in the products of some representative fermentations was determined by gas liquid chromatography and compared with VFA composition in the rumen fluid. As shown in Table VI, molar proportions of VFA in the rumen [18] of hay fed cattle are

TABLE VI

VFA Composition of Product Produced by Fermentation of FLWF or Manure by Natural Flora[1]

Treatment	Sample	VFA[4] (umoles/ml) A	P	B	Ratio A : P : B
FLWF[2] + 5% Whey Powder	Initial	25.7	2.8	-	
	Final	295.7	310.0	22.8	46:48: 3
FLWF + 5% Corn Starch	Initial	55.7	7.1	-	
	Final	401.4	105.7	32.8	74:19: 6
FLWF + 5% Potato Starch	Initial	67.4	10.0	1.4	
	Final	310.0	1.4	71.4	81:.3:19
Manure[3] + 5% Whey Powder	Initial	17.8	1.4	0.7	
	Final	121.4	118.6	55.7	38:37:17
Manure + 5% Whey Powder + L. bulgaricus	Final	150.7	174.3	45.4	38:43:12
Hay Ration-Rumen[5]					66:18:12
Concentrate Ration-Rumen[5]					46:32:18

[1] Fermentations were run at 40°C and pH 5.5 for 24 hr. pH adjusted to 6.8 at the end of fermentation. Fermentations were nonsterile and no additional inoculum was added unless otherwise indicated.

[2] FLWF refers to FLW diluted 1:1 with water and filter-pressed through two layers of cheesecloth.

[3] Manure was diluted 1:9 with water before fermentation.

[4] VFA stands for volatile fatty acids; A = acetate; P = propionate; B = butyrate.

[5] These values were adapted from Hungate [18].

67:18:12 (acetate:propionate:butyrate) and in animals on a high grain diet are 46:32:18 (acetate:propionate:butyrate). Note the high acetic acid and low propionic acid levels in hay fed animals, and a proportionately higher propionic acid and lower acetic acid in grain fed animals. High molar proportion of propionic acid in the rumen is correlated with higher feed efficiency and faster weight gains in feedlot cattle. Also Allen et al. [17] showed that feedlot cattle fed ammonium propionate had a higher feed efficiency than animals fed ammonium salts of acetate, butyrate, lactate, or urea. It is, therefore, of interest that in FLWF fermentations supplemented with whey, the product obtained has a much higher molar proportion of propionic acid than was recorded for fermentations in the rumen. This is a very desirable and important feature of this fermentation. VFA accounted for greater than 90% of the carbohydrate metabolized. Valerate, lactate, and succinate are produced in trace amounts.

It is also of interest that FLWF fermentation supplemented with corn starch has a much lower concentration of propionic acid in the product, while FLWF supplemented with potato starch has only trace amounts of propionic acid in the product. This suggests a shift in microbial population in FLWF fermentation supplemented with starchy materials as compared to the same fermentation supplemented with whey.

Also, note that the fermentation of fresh manure diluted 1:9 with water and supplemented with whey, and either receiving or not receiving an inoculum of *L. bulgaricus* at the 10% level, yields a product that has a similar acetate:propionate ratio as the product obtained by fermentation of FLWF plus whey. However, the manure product has significantly higher butyrate content. Nonvolatile acids (lactate and succinate) were present only in trace amounts.

The results also indicate that in different fermentations the total VFA produced accounts for 72% to more than 90% of the carbohydrate metabolized. The reason for this wide variation may be due to one or more of the following reasons: 1) qualitative and/or quantitative variations in microbial population in different batches; 2) changes in microbial population brought about by the type of supplemental carbohydrate used; 3) differences in degrees of efficiency with which different carbohydrates are metabolized, and 4) differences in metabolic pathways used for the utilization of different substrates.

CONCLUSIONS

Feedlot waste filtrate (FLWF) is low in carbohydrate and it is not practical to ferment FLWF without supplemental carbohydrate added.

FLWF obtained from 1:1 or 1:9 diluted FLW could be supplemented with other agricultural wastes, rich in carbohydrates, and fermented successfully to yield a product which when concentrated fivefold contains 20% or higher amount of crude protein. Supplementation of FLWF with whey, molasses, starch from potato processing wastes, or corn starch will yield a

product similar in crude protein content, but significantly different in VFA content.

The fermentation process developed is simple and efficient and has several advantages.

1) The process is anaerobic, thus eliminating high equipment costs involved in aeration. Yet, no special anaerobic conditions need be provided. Most of the substrate carbon is conserved as volatile fatty acids that are excellent sources of energy to the ruminant animal.

2) There is no requirement for supplementation with growth factor sources and separate inoculum need not be added. There is no need for sterilization of the medium or observing other aseptic conditions, and the product is fairly uniform.

3) Added carbohydrates are rapidly fermented (24 hr).

4) The process is simple and easily adaptable to existing industrial technology and does not require any specially trained individuals for the actual operation.

5) The product produced is stable and is not subject to microbial spoilage for several months when stored at 25°C.

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SECTION 2 (ARTICLE 2)

OPTIMIZATION OF A BATCH PROCESS FOR THE FERMENTATIVE CONVERSION OF CHEESE WHEY-SUPPLEMENTED CATTLE FEEDLOT WASTE FILTRATE INTO A NITROGENOUS FEED SUPPLEMENT FOR RUMINANTS

By

M.D. Erdman and C.A. Reddy

ABSTRACT

Optimization of a batch process for the fermentative conversion of cattle feedlot waste filtrate (FLWF), supplemented with cheese whey, into a nitrogenous feed supplement for ruminants is described. FLWF plus cheese whey powder (5 g/100 ml) combination was fermented for 6 - 8 h by the indigenous microbial flora in the FLWF at 43°C. The pH was maintained constant at 7.0 during the fermentation by the automatic addition of ammonia. Ammonium salts of organic acids produced are valuable as nitrogenous feed supplements to ruminants. Under these conditions, the percent utilization of substrate carbohydrate was > 94% within 8 h. The crude protein (total N x 6.25; CP) content of the product was 70 - 78% (dry basis). About 66 - 69% of the CP in the product was in the form of ammonia nitrogen (AN). After 8 h of fermentation at pH 7.0, lactate and acetate were the predominant acids, but at the end of 24 h appreciable levels of propionate and butyrate were also present. The rate of fermentation and CP content of the product were optimal at pH 7.0 and decreased at lower pH: 4.5 < 5.0 < 5.5 < 6.0 < 6.5 < 7.0; fermentation did not go to completion even after 24 h at pH 4.5. Fermentation at pH 7.5 was almost as rapid as that at pH 7.0. The fermentation proceeded optimally at 43°C, less so at 37°C and was considerably slower at 23° and 50°C. Cheese whey concentrations up to 15 g/100 ml of FLWF were fermented efficiently. Fermentation of FLWF obtained from animals fed a low silage-high grain, high silage-low grain or dairy ration

resulted in a similar product.

INTRODUCTION

Cattle wastes account for about 70% of the > 1.8×10^{12} kg of livestock wastes produced annually in the United States (4,22). Nearly 50% of these wastes are generated in confined animal production systems such as cattle feedlot operations (41). Disposal of such large volumes of feedlot waste (FLW) represents a growing and critical problem with respect to environmental pollution, wastage of large volumes of potentially utilizable nutrients (2×10^9 kg of total nitrogen) and a financial drain to the livestock industry (4,7,24,31, 33,41). However, FLW can also be considered as a valuable renewable resource, if utilized properly. For example, recycling FLW as a livestock feed ingredient would partially alleviate the disposal problem and supplement our dwindling food/feed resources. The on-site concentration of FLW, which allows continuous collection and processing, is a distinct advantage in this respect. A number of reviews (1,2,3,6,20, 34,35,41) and reports relative to the use of unfermented (13,23,25,36,37), ensiled (2,16,28) or aerobically fermented (5,9,17,21,27,32,39,40) FLW as a livestock feed have been published.

Reddy and Erdman (31) recently described a novel, anaerobic, ammoniated organic acid fermentation process (15) for conversion of cattle feedlot waste filtrate (FLWF) into a potentially useful nitrogenous feedstuff for ruminants. In this process, FLWF, which contains 72% of the total

nitrogen in FLW and is equivalent to casein in amino acid composition but is low in carbohydrates (33), was supplemented with a complimentary carbohydrate-rich agricultural waste as exemplified by cheese whey (30) or starch recovered from potato processing wastes (14). This combined substrate was then fermented batchwise, anaerobically, by indigenous microbiota in FLW at 43°C and pH 5.5 for 24 h. The objective of the fermentation was to maximize the conversion of carbohydrate in the substrate to organic acids and to neutralize the latter with ammonia to produce ammonium salts of organic acids. These acids were generally shown to be superior to urea and comparable to soybean meal as nitrogenous feed supplements to ruminants (reviewed in ref. 15). A number of advantages inherent in this approach, as compared to processes for single-cell protein production from wastes, were summarized (31).

The purpose of the present investigation was to study the effect of pH, temperature and cheese whey concentration on the rate of fermentation, and on the rates and types of organic acids produced during the fermentation, and to define optimal operating conditions for the batch FLWF fermentation. (This paper was presented in part at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada, May 14 - 19, 1978.)

MATERIALS AND METHODS

Substrate. Unless otherwise stated, fresh ($\leq$ 1 day old) FLW, collected from finishing steers fed a high-silage ration

(88% corn silage, 12% soybean meal), was used throughout this study. The FLW, which contained on the average 16.5% total solids (TS), was diluted 1:1 (w/w) with water, mechanically agitated for 30 min and filtered through a screen (3 mm^2 mesh size) to remove large particulate material. The resulting feedlot waste filtrate (FLWF) contained 7 - 9% TS, or approximately 18% of the TS in FLW. The FLWF was supplemented with 5 g/100 ml of cheese whey powder (CWP; Michigan Milk Producers, Ovid, MI). The CWP contributed 0.07 g of TN and 3.83 g of lactose/100 ml of FLWF and was used as the fermentation substrate except where otherwise indicated.

Fermentation procedures. Laboratory scale anaerobic 20-liter batch fermentations were performed as previously described (12) except for the following modifications. Fermentations were conducted at pH 7.0 and 43 $\pm$ 0.5°C unless otherwise indicated. Indigenous microbiota in FLWF served as the inoculum. No special sterile or anaerobic precautions were taken except that the fermentor was closed to the outside environment.

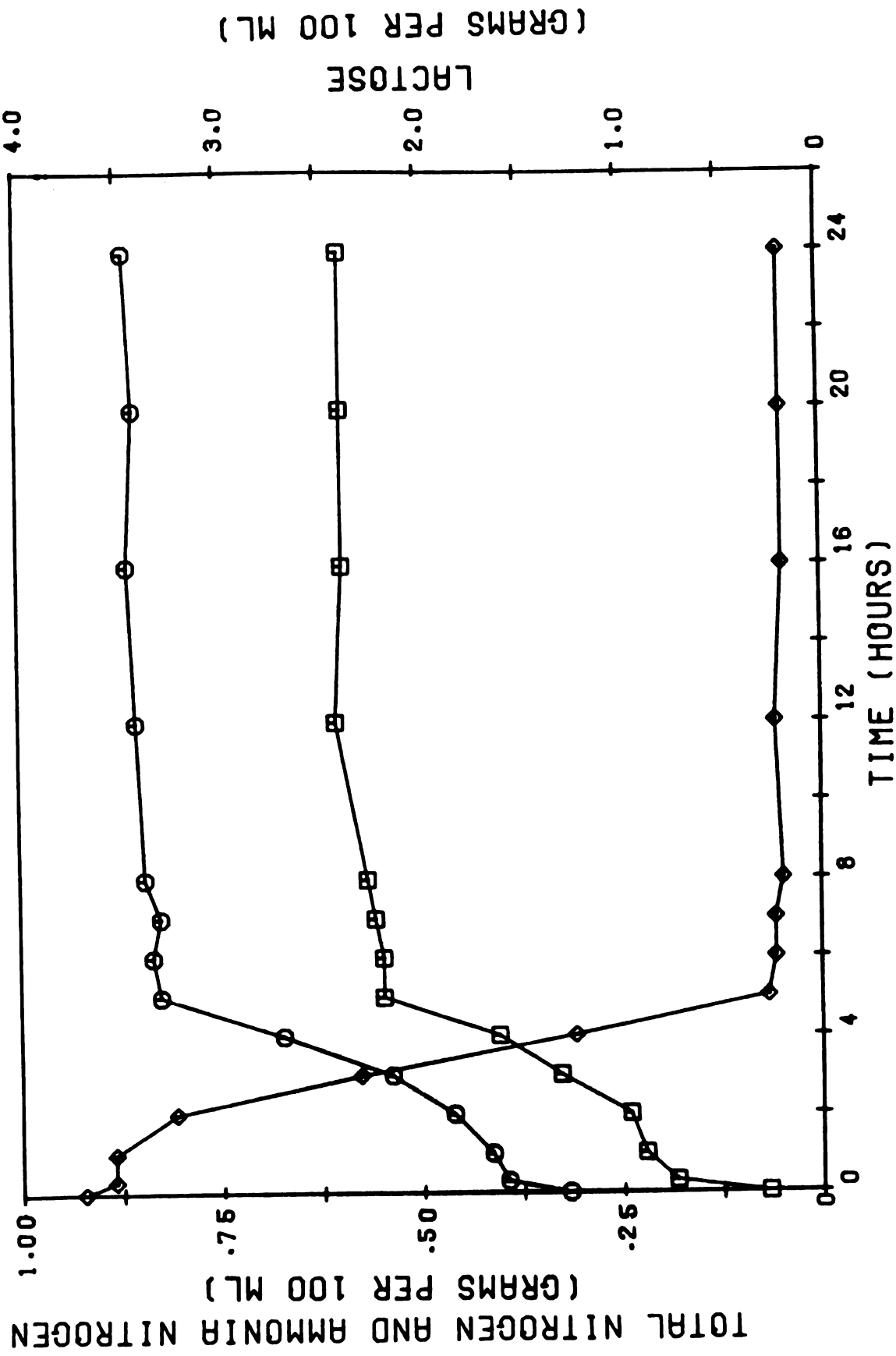
Analytical procedures. Samples ($\sim$ 80 ml) were collected in glass screw-capped bottles at various times during the fermentation and stored at -18°C until analyzed. All samples were analyzed for TN, ammonia nitrogen (AN), lactose, TS, and volatile and non-volatile acids. The TN concentrations of whole samples were determined by the micro-Kjeldahl procedure(38). The TS were determined gravimetrically by drying

50-70 g samples at 50°C and reduced pressure to constant weight. The supernatant obtained after centrifugation (20,000 x g for 30 min at 4°C) of the samples was used for determining the concentrations of volatile (VFA) and non-volatile acids (NVA) by a gas chromatograph (Model 5730A, Hewlett-Packard) equipped with a flame ionization detector and temperature programming. VFA concentrations were determined using a column packing of 3% carbowax, 20 M/0.5% H_3PO_4 on 60/80 carbopack B (Supelco, Inc., Bellefonte, PA) by the procedure of Dicorcia and Samperi (10) except that the initial column temperature of 100°C was raised (4°C/min) to 200°C. NVA were esterified and extracted by the procedure of Holdeman and Moore (19). Extracts were injected onto a column of 10% SP-1000/1% H_3PO_4 on 100-120 Chromosorb W AW (Supelco). The initial column temperature of 90°C was raised (4°C/min) to 130°C. For the determination of lactose (11,26) and AN (29),5.0 ml of supernatant was treated with 1.0 ml of 1.96 M 5-sulfosalicylic acid (SSA) and incubated at ambient temperature for 30 min. Samples were then diluted (1:1), centrifuged at 20,000 x g for 30 min and the clarified supernatants were used for analysis.

RESULTS

Rate study. The rate of decrease in lactose concentration and rates of increase in concentrations of AN and TN during the fermentation at 43°C are shown in Fig. 1. Most of the whey lactose (> 94%) was metabolized within 6 h. As lactose

Figure 1. Rate of lactose utilization (-◇-), and rates of increase in ammonia nitrogen (-□-) and total nitrogen (-○-) concentrations during the fermentation of FLWF, supplemented with 5.0 g CWP/100 ml, at 43°C and pH 7.0.


LACTOSE
(GRAMS PER 100 ML)
4.0
3.0
2.0
1.0
0
TOTAL NITROGEN AND AMMONIA NITROGEN
(GRAMS PER 100 ML)
1.00
.75
.50
.25
0
0
4
8
12
16
20
24
TIME (HOURS)

concentration decreased, there was a proportional increase in concentrations of AN and TN. The content increased 2.6 fold during the fermentation; the product contained 70 - 78% CP on a dry basis. AN accounted for 66 - 69% of the TN. The data show that the progress of the fermentation in the first 6 - 8 h could be monitored efficiently by measuring either a decrease in concentration of lactose or the increase in concentration of AN or TN. Furthermore, > 90% of the increase in TN was accounted for by the increase in AN.

Effect of pH. The effect of pH upon fermentation, as measured by the rate of increase in TN and the rate of lactose utilization, are presented in Figs. 2A and 2B, respectively. The rate of increase in TN (Fig. 2A)was directly related to increasing pH. Fermentations maintained at pH 7.0 and 7.5 gave the fastest rate of TN increase and were essentially complete within 6 and 8 h, respectively. Fermentations maintained at pH 4.5 and 5.0 had the slowest rate of TN increase. Maximum rate of lactose utilization (Fig. 2B) was observed at pH 7.0 although somewhat similar rates were observed at pH 6.5 and 7.5. However, pH 7.0 was considered optimal because greater amounts of supplemental lactose were metabolized at this pH and nitrogen losses in this product during storage were lower than in that produced at pH 7.5 (results not shown).

Organic acid composition was affected rather dramatically by pH and time of fermentation (Figs. 3A-3G). At pH 7.0 (Fig. 3F) lactate and acetate were the predominant acids at

Figure 2. Effect of pH 4.5 (-⬡-), 5.0 (-□-), 5.5 (-◇-), 6.0 (-△-), 6.5 (-✡-), 7.0 (-✶-) and 7.5 (-✳-) upon total nitrogen increase (A) and lactose utilization (B) during fermentation of FLWF. Fermentation conditions as in Figure 1, except for pH.

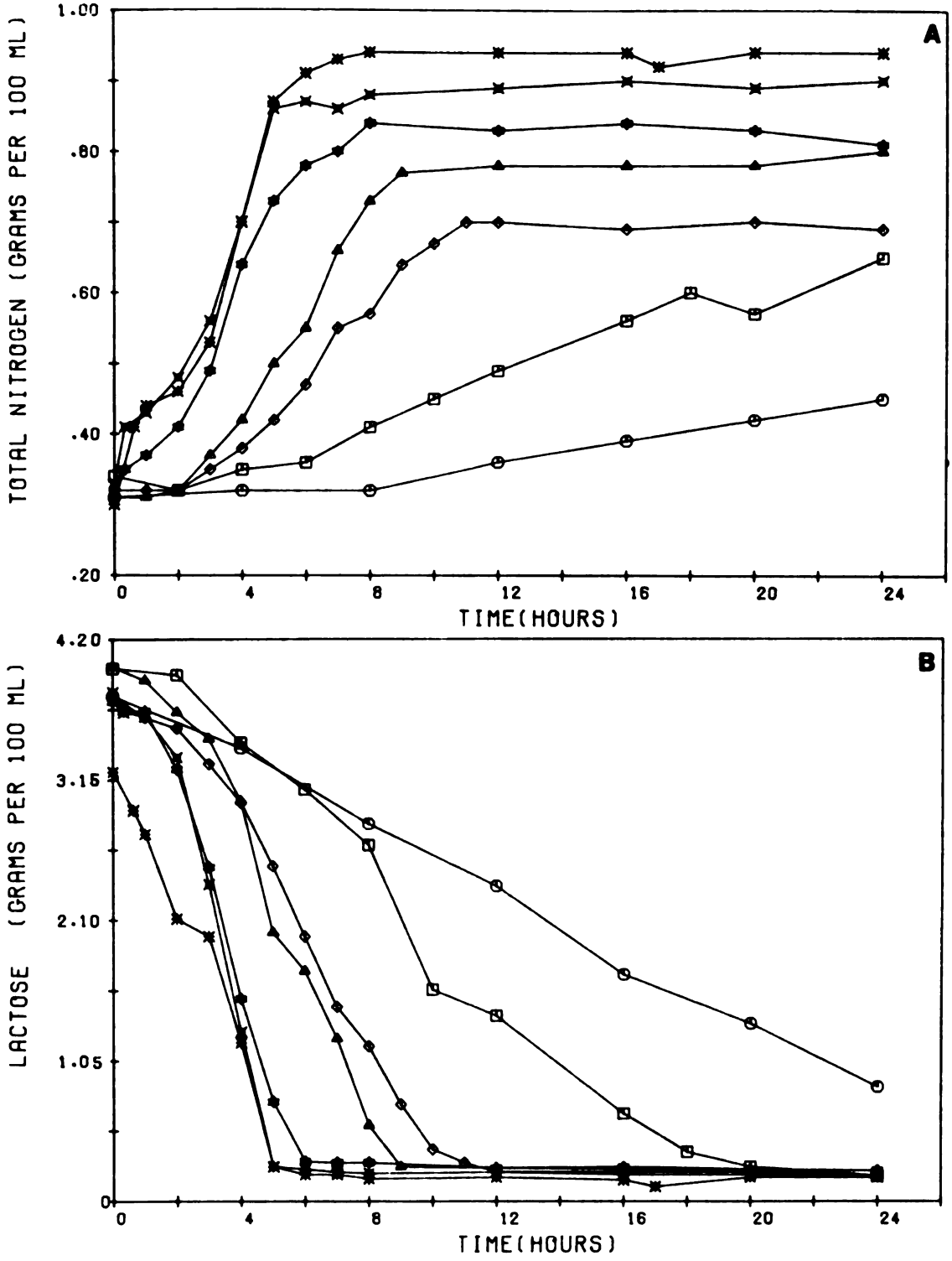
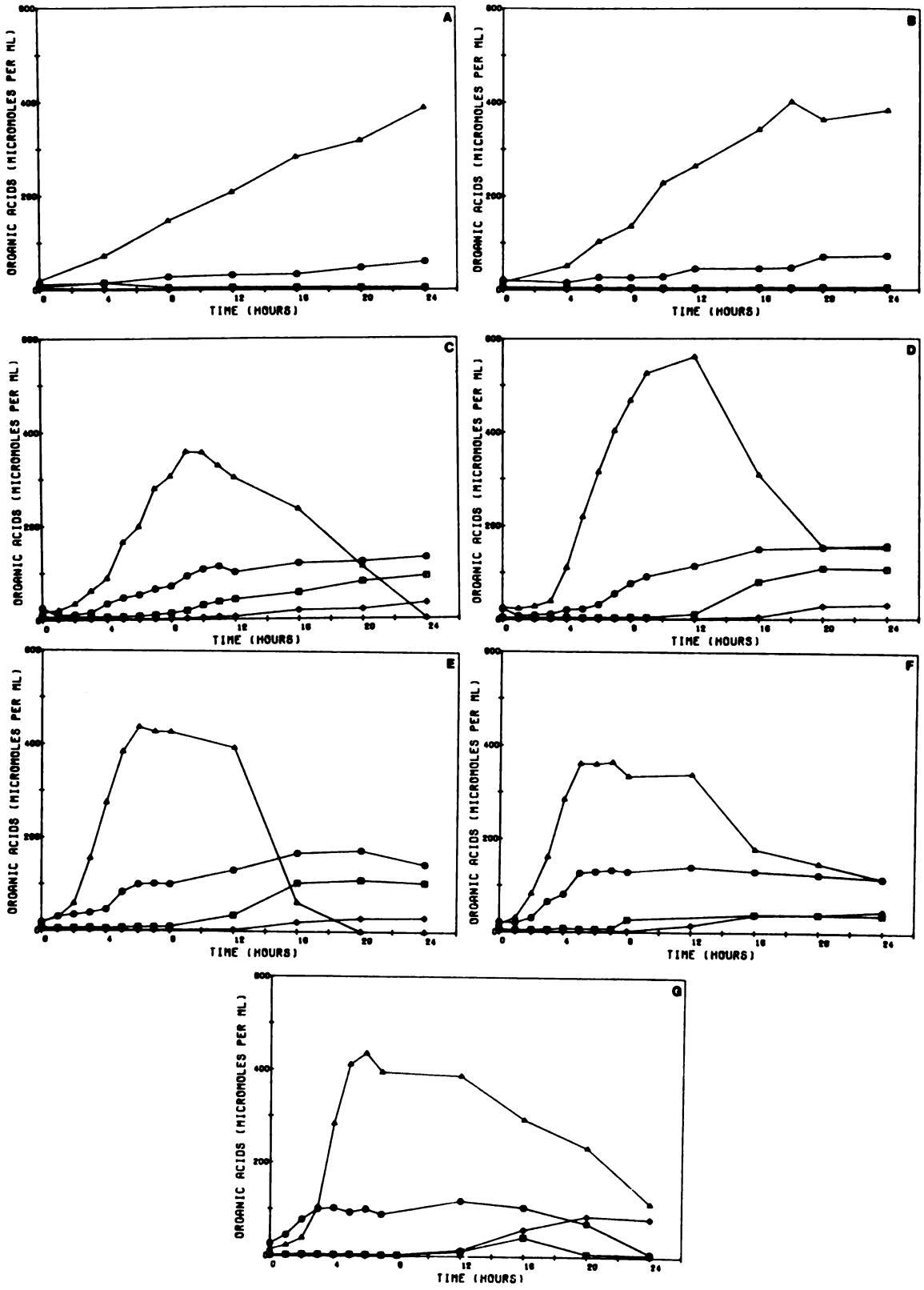

A
TOTAL NITROGEN (GRAMS PER 100 ML)
1.00
.80
.60
.40
.20
0
4
8
12
16
20
24
TIME(HOURS)
B
LACTOSE (GRAMS PER 100 ML)
4.20
3.15
2.10
1.05
0
0
4
8
12
16
20
24
TIME(HOURS)

Figure 3. Effect of pH 4.5 (A), 5.0 (B), 5.5 (C), 6.0 (D), 6.5 (E), 7.0 (F) and 7.5 (G) on concentrations of organic acids with time during fermentation of FLWF; other experimental conditions as in Figure 1. Symbols: ⬡, acetic; □, propionic; ◇, butyric; and △, lactic.


A
B
C
D
E
F
G
ORGANIC ACIDS (MICROMOLES PER ML)
TIME (HOURS)

the end of 6 - 8 h (72:26, respectively) but lactate, acetate, propionate and butyrate were predominant at the end of 24 h (37:23:3:37, respectively). Somewhat, similar results were obtained with fermentations conducted at pH 5.5,6.0 and 7.5 (Fig. 3C,D, and G). In contrast, the fermentations were essentially of the homolactic type (8) at pH 4.5 (Fig. 3A) and 5.0 (Fig. 3B).

Effect of temperature. The data presented in Fig. 4 show the effect of temperature on the rates of increase in TN during fermentation. Optimum fermentation, as determined by most rapid increase in TN and the highest TN concentration at the end of 5 h, occurred at 43°C and the fermentation was essentially complete in 5 h while fermentation maintained at 37°C required 8 - 10 h for completion. Fermentations at 23°C and 50°C were incomplete even at the end of 20 h. When fermentation was initiated at ambient temperature (23°C) and the fermentation temperature was not controlled, there was an initial lag period of about 8 h, but fermentation was complete in 20 h. Temperature of the fermentation broth rose from 23°C at zero time to 33°C at the peak of fermentation (18 -20 h), indicating that heat was generated during fermentation.

The effects of temperature on concentration of organic acids with time during the fermentation are shown in Figs. 5A and 5B. In fermentations in which the temperature was not controlled (Fig. 5A), there was marked initial lag followed

Figure 4. Effect of temperature on rate of increase in TN during fermentation of FLWF, supplemented with cheese whey, maintained at pH 7.0; other experimental conditions as for Fig. 1. Symbols: ⬡, temperature not controlled; ✿, 23 C; □, 37 C; ◇, 43 C; △, 50 C.

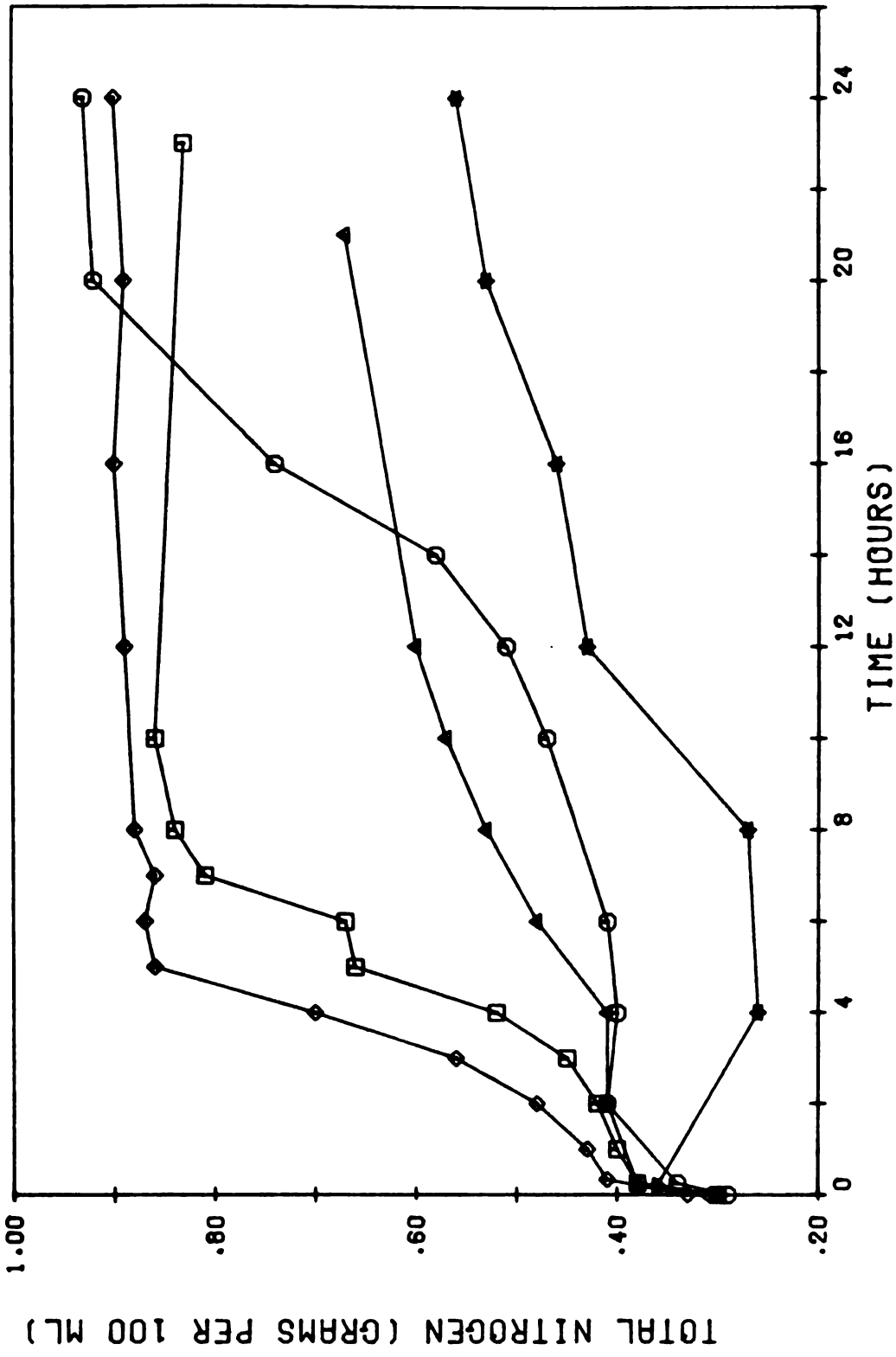
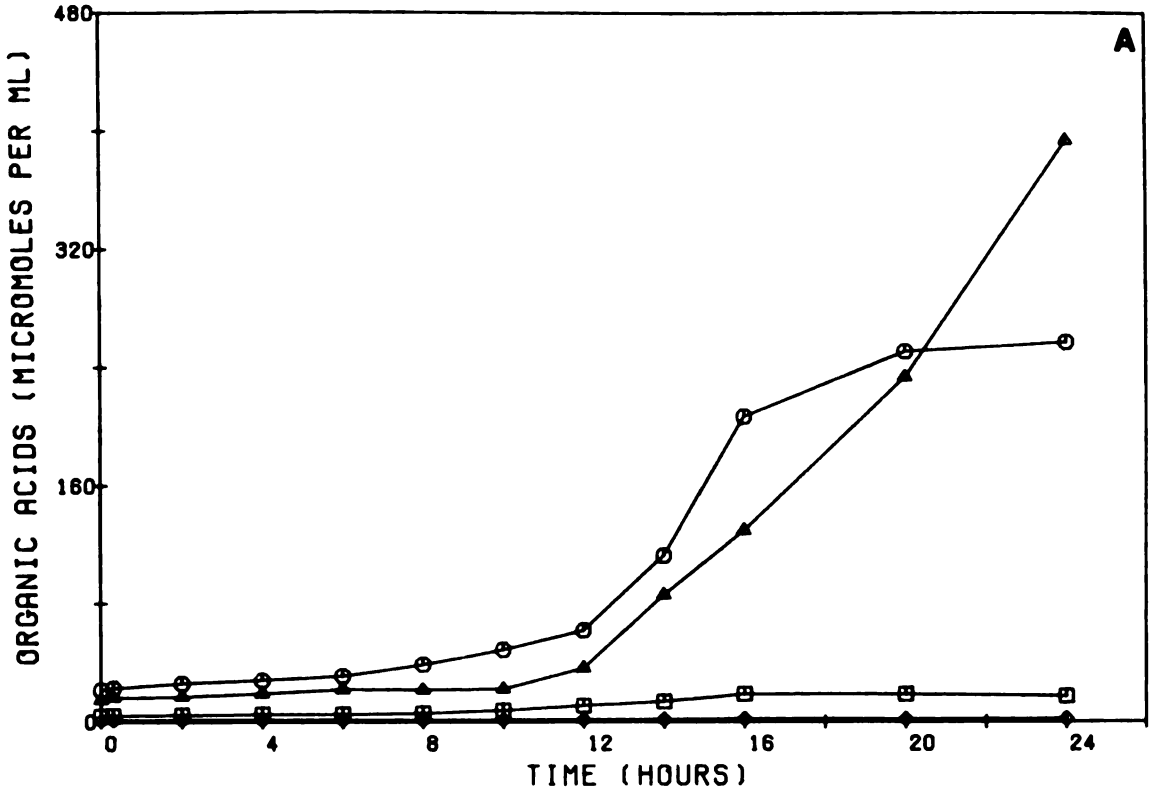
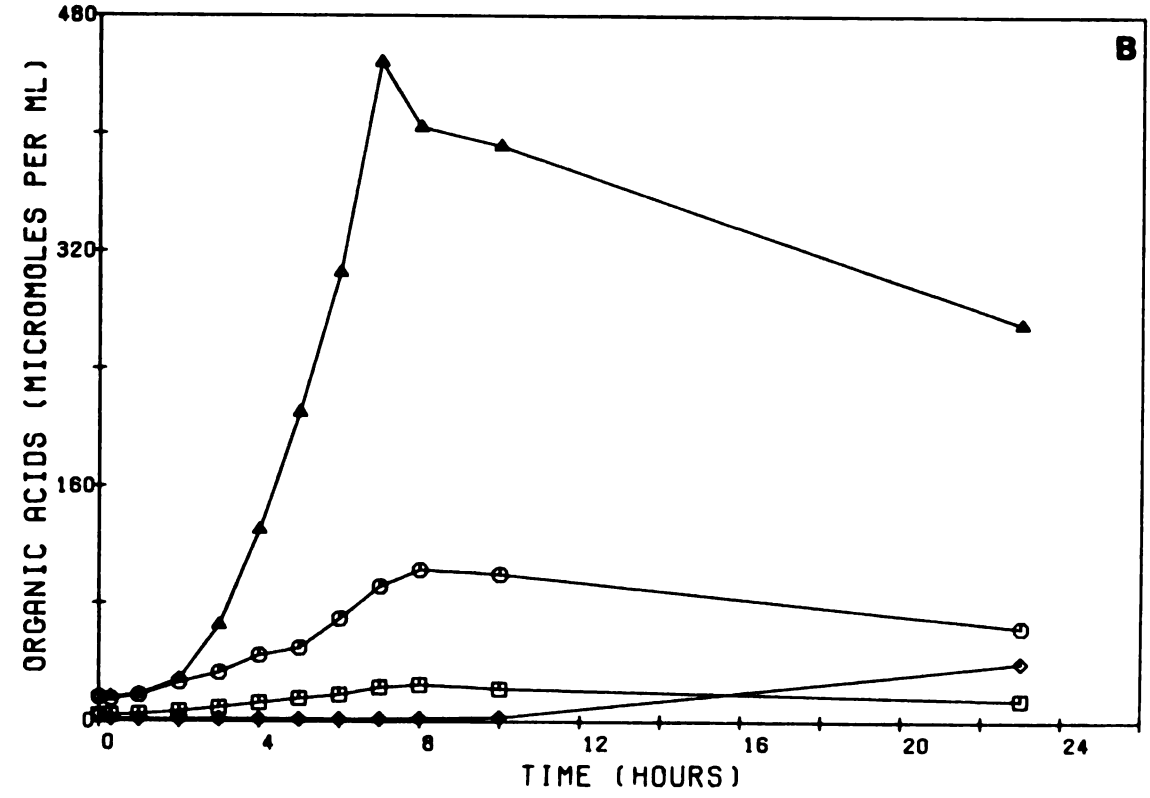

TOTAL NITROGEN (GRAMS PER 100 ML)
1.00
.80
.60
.40
.20
0
4
8
12
16
20
24
TIME (HOURS)

Figure 5. Effect of temperature on organic acids concentrations (acetic, ⬡ ; propionic, □ ; butyric, ◇ ; lactic, △) with time during fermentation of FLWF supplemented with 5.0 g CWP/100 ml and maintained at pH 7.0. Temperature not controlled (A) or controlled at 37°C (B). See Fig. 3F for similar data on fermentation conducted at 43°C.

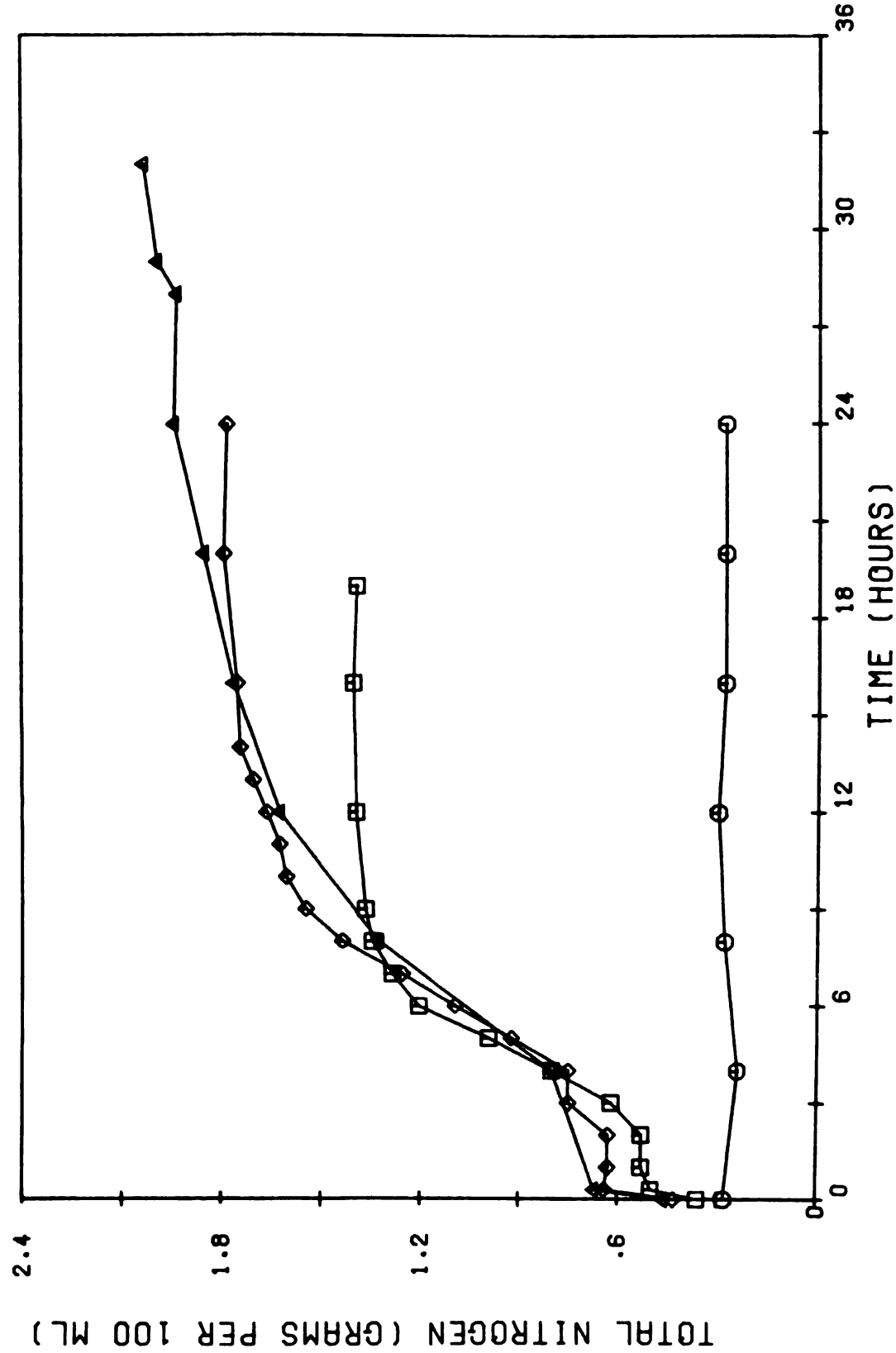

A
480
320
160
0
ORGANIC ACIDS (MICROMOLES PER ML)
0
4
8
12
16
20
24
TIME (HOURS)


B
480
320
160
0
ORGANIC ACIDS (MICROMOLES PER ML)
0
4
8
12
16
20
24
TIME (HOURS)

by rapid increases in the concentrations of acetate and lactate. At the end of 24 h, acetate and lactate accounted for 38 and 58% of the total acids, respectively. In fermentations maintained at 37°C (Fig. 5B), lactate increased rapidly and reached a peak at 7 h and then decreased considerably by the end of 23 h. A decrease in lactate concentration between 7 and 23 h was correlated with an increase in butyric acid concentration during this period. Acetate concentration increased through 8 h of fermentation and remained relatively stable thereafter. At the end of 24 h, lactate,acetate, propionate and butyrate accounted for 67.8,16.0,3.7, and 10.1% of the total acids, respectively. In contrast, lactate, acetate, propionate and butyrate accounted for 36.2, 37.3, 11.8 and 13.2% of the total acids, respectively, after 24 h of fermentation at 43°C (Fig. 3F). Fermentations maintained at 23°C contained 31.7 and 202.8 μmoles of total acids/ml at '0' and 24 h, respectively; lactate and acetate accounted for 75.7 and 19.3% of the total acids, respectively, at the end of 24 h (results not shown). In fermentations maintained at 50°C, total organic acids concentration was 195.2 μmoles/ml at 24 h and acetate and butyrate accounted for 66.6 and 31.1% of the total acids, respectively (results not shown).

Effect of initial concentrations of CWP. At initial CWP concentrations of 5% (Fig. 1) or 10% (Fig. 6) the fermentation proceeded rapidly and essentially went to completion in 8 h. When CWP was added at the 15% level, 16 h were required for completion of the fermentation whereas at 20%

Figure 6. Effect of CWP concentration on rate of increase in total nitrogen during fermentation of FLWF maintained at pH 7.0 and 43°C. Symbols: ⬡, 0.0 g; □, 10.0 g; ◇, 15.0; or △, 20.0 g CWP/100 ml FLWF.

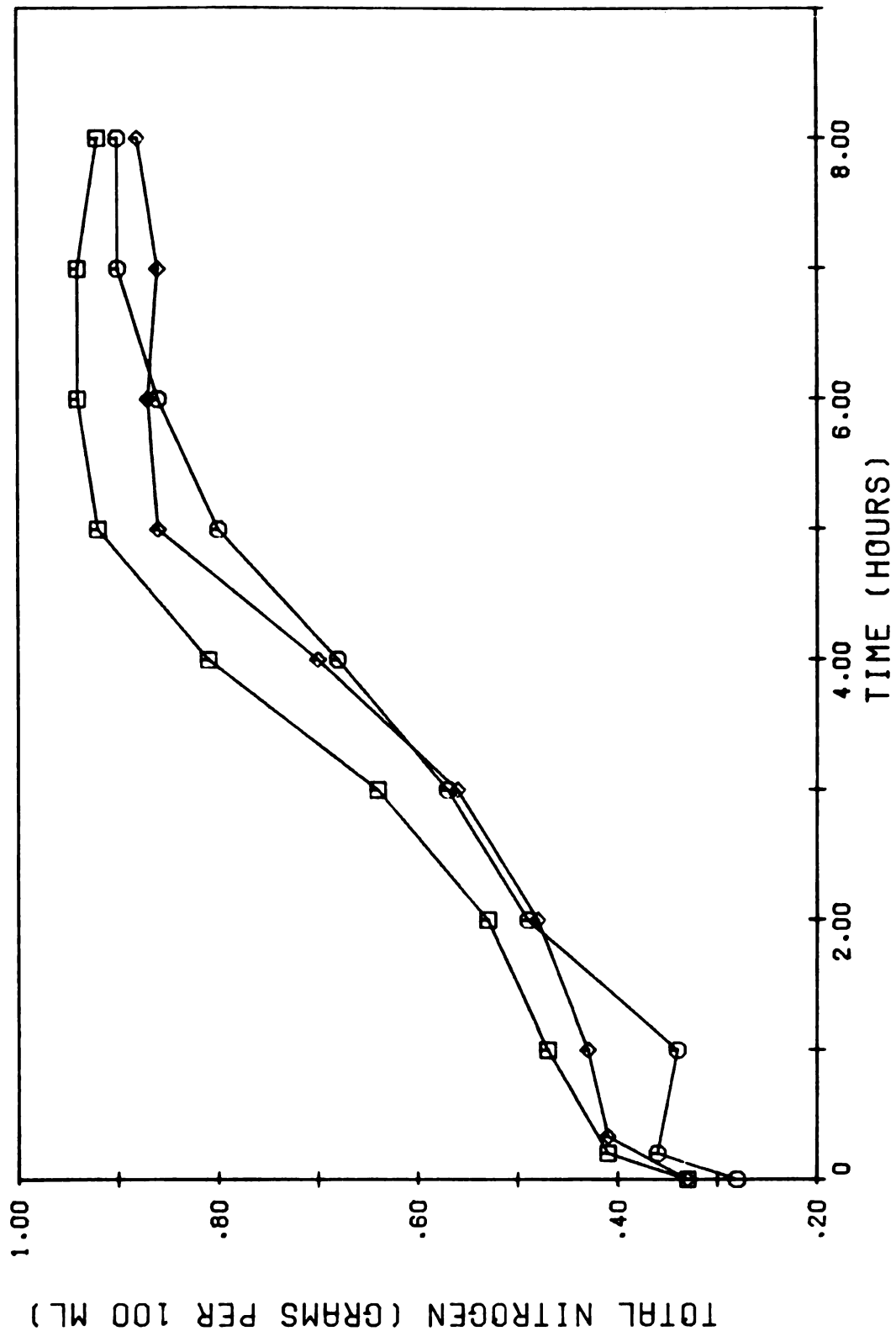

TOTAL NITROGEN (GRAMS PER 100 ML)
2.4
1.8
1.2
.6
0
0
6
12
18
24
30
36
TIME (HOURS)

initial concentration of CWP, fermentation of lactose was incomplete even at the end of 32 h. Lactate and acetate were the predominant acids (78.8 and 18.6%, respectively) during fermentations supplemented with 10% CWP. Similar results were obtained during fermentation of FLWF, supplemented with 15% or 20% CWP. In fermentations receiving 0,5,10, 15 and 20 g CWP/100 ml, the total organic acids concentrations at 24 h (μmoles/ml) were 40,501,1000,1314 and 1040, respectively.

Effect of ration composition. The objective of this experiment was to determine the variation, if any, in fermentation rate and product composition when FLWF, collected from animals fed different rations, was used as the substrate. No appreciable differences were observed in the rates of fermentation, as determined by the rate of increase of TN, when FLWF from animals fed the three different rations was fermented (Fig. 7). The TN and AN content, and the rate of production and composition of organic acids were similar for all three fermentations (results not shown).

Effect of manure storage. In this experiment, fermentation of FLWF derived from fresh manure versus that derived from manure stored for 91 days outside at ambient temperature in late Fall was compared. Each FLWF was supplemented with 5% CWP and fermented individually at 43°C and pH 7.0. The products obtained in both cases were similar in TN and AN content. However, 20 h were required for completion of the fermentation when FLWF from stored manure was used. When FLWF derived from stored manure was supplemented with

Figure 7. Increase in TN with time when FLWF from animals fed different rations was fermented, after augmentation with CWP at 5 g/100 ml. Other experimental conditions as described in the legend for Fig. 1. Symbols: high silage, -◇- ; high grain, -□- ; dairy, -⬡- .


TOTAL NITROGEN (GRAMS PER 100 ML)
1.00
.80
.60
.40
.20
0
2.00
4.00
6.00
8.00
TIME (HOURS)

10% fresh FLWF, the fermentation time and the product obtained were similar to that observed with fresh FLWF.

DISCUSSION

The results conclusively showed that ammoniated organic acid fermentation of FLWF supplemented with CWP at 5% or 10% level could be optimally carried out batchwise at pH 7.0 and 43°C using indigenous microbial flora as the inoculum. Under these conditions, fermentation of the added substrate was essentially complete in 6 - 8 h. This optimized batch FLW process is thus about 3 - 4 fold more rapid than the original process described by Reddy and Erdman (31). Also, the process described here is > 6 - 12 times faster and the crude protein content of the product much higher than previously described aerobic fermentation of FLWF utilizing fungi and streptomycetes (40), *Trichoderma viride* (17,21, 27), or indigenous microbiota in FLW (32,39).

FLWF is known to contain 70 - 80% of the TN orginally present in FLW (33). The process described here thus allows recycling of approximately 20% of the TS and 70 - 80% of the TN originally present in FLW while at the same time recycling a complimentary waste carbohydrate source such as cheese whey. The two wastes (FLWF and cheese whey) complemented deficiencies in nitrient composition in each other. It is most likely that the primary contribution of cheese whey to the fermentation is lactose, while FLWF provides inoculum, growth factors required by the microbiota and some proteins. For example, FLWF is low in readily fermentable carbohydrate and, therefore, fermentation of unsupplemented FLWF results in no appreciable increase in TN (31). In constrast, fermentation of FLWF supplemented with cheese whey resulted in a product with

high TN content. Although we utilized CWP as a convenient carbohydrate source in this study, we conducted several laboratory scale fermentations in which FLWF was supplemented with fresh acid cheese whey or deproteinized whey, instead of CWP, and obtained comparable results (Erdman et al. unpublished data). Furthermore, this fermentation has been scaled up to a 2200 - liter pilot plant fermentor, utilizing fresh acid cheese whey as the carbohydrate supplement and diluent, and the product produced is being tested for its nutritional efficacy as a nitrogenous feed supplement to beef steers.

The pH of fermentation appear to greatly affect rates of production, concentration and composition of organic acids, suggesting dynamic changes in microbial populations and product quality occurring throughout the fermentation. For example, at pH 4.5 and 5.0, the fermentations were essentially of the homolactic type,whereas at pH 5.5, 6.0, 6.5,7.0 and 7.5, mixed acid type fermentations were observed. In fermentations maintained at pH 5.5 through 7.5, lactate and acetate were the predominant acids after 6 - 8 h of fermentation; thereafter, lactate appeared to undergo secondary metabolism as demonstrated by the rather dramatic decrease in lactate concentration and increases in the concentration of propionate and butyrate. Furthermore, butyrate production appeared to be more prominent at pH 7.5 than at other pH's examined; also, acetate and propionate appeared to undergo secondary metabolism after 16 h and were not detectable at 24 h at pH 7.5. It is significant that there was no increase in TN concentration between 8 - 24 h of fermentation at 43°C, pH 7.0 - 7.5. It is, therefore, desirable to terminate the fermentation between 6 - 8 h when the concentration of total organic acids, primarily lactate, is the highest. The nutritional value of

ammonium lactate for ruminants has been well documented (18). Also, termination of the fermentation prior to secondary metabolism of the organic acids, permits conservation of most of the carbon in the product as fermentation acids which are valuable sources of energy for ruminant animals.

As optimum fermentation occured at 43°C, fermentation at this temperature would allow for maximum utilization of FLW in minimum time. This may be important at sites where the magnitude of FLW accumulation is high. However, if large fermentor capacity and/or relatively limited amounts of FLW was available (all other things being equal), it may be more desirable to ferment FLWF without any temperature control to save energy and production costs, at least as long as the ambient temperature is not below 20°C.

The process described and optimized is an attractive alternative to disposal of two important agroindustrial wastes. It is simple, efficient and easily adaptable to processing of FLW at the source. The process is anaerobic, thus eliminating high equipment costs involved in aeration; yet no special anaerobic conditions need be provided. Most of the substrate carbon is conserved as fatty acids which are good sources of energy to the ruminant. There is no need for sterilization of the medium, no requirement for supplementation with growth factor sources and separate inoculum need not be added; thus, further reduction in operating costs are achieved. Preliminary results show that the product, after stabilization with formaldehyde (Reddy and Meitz, unpublished data) can be fed directly as nitrogenous feed supplement to ruminant animals (Yokoyama et al. unpublished data).

The potential for health (animal and human) problems related to the recycling of animal wastes by feeding has been discussed by

Fontenot and Webb (13). Samples of fermentation product produced during this investigation were tested for the presence of pathogens throughout the investigation by the Veterinary Clinical Diagnostic Laboratory at Michigan State University. No aerobic or anaerobic pathogens were detected. Furthermore, when 10^5 Salmonella sp./ g of fermentor contents were added at 0 h, no viable Salmonella could be recovered at the end of 24 h of fermentation (Reddy and Meitz, unpublished data). These and other results (39,40) suggest that feeding fermented feedlot waste to cattle does not introduce risk to animal and/or human health. However, more detailed investigations are needed to conclusively establish the safety of this product.

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SECTION 3 (ARTICLE 3)

A CONTINUOUS FERMENTATION PROCESS FOR RECYCLING FEEDLOT WASTE FILTRATE AS A RUMINANT FEED SUPPLEMENT

By

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SUMMARY

A continuous fermentation process for the conversion of two types of complementary agricultural residues, feedlot waste filtrate and whey lactose, into a protein feed supplement for ruminants is described. Cattle feedlot waste, diluted 1:1 with water, was separated into filtrate (FLWF) and fiber fractions in a pneumatic slurry separator. FLWF, supplemented with 5 g/100 ml cheese whey powder (CWP), was fermented continuously at 43 C and pH 7.0 under semi-anaerobic conditions. Indigenous flora served as the inoculum. Ammonia was added continuously and automatically during the fermentation to maintain a constant pH and to produce ammonium salts of organic acids which are valuable as crude protein supplements to ruminant animals. At the optimal retention time of 1.9 h (as determined by maximum total nitrogen value), the residual lactose concentration was 0.17 g/100 ml, i.e. 96% of the added lactose was metabolized. The rate of lactose utilization was 19.5 mg/ml/h. The rate of lactose utilization was inversely proportional to the retention time. Organic acids accounted for > 95% of the lactose utilized. The product had 70-75% crude protein on a dry basis. Ammonia nitrogen constituted 65-70% of the total crude protein in the product. Lactate was the predominant acid at shorter retention times; at higher retention times lactate concentration decreased considerably and the concentration of acetic, propionic and butyric acids gradually increased. The optimal retention time of 1.9 h was 3-4 times shorter than the time required for

optimal batch fermentation of an identical substrate. When FLWF was supplemented with 10 and 15% CWP, optimum retention times were 7.7 and 10.4 h, respectively; however, crude protein content (dry basis) in the product was considerably lower at higher than 5% levels of cheese whey supplementation. Amounts of CWP added to FLWF, and retention times greatly influenced the organic acid composition in the product. The results showed that the continuous process described here for the recycling of FLWF is considerably more efficient in terms of fermentation time and lactose utilization than previously described processes. The process described provides an attractive alternative approach to conventional waste disposal methods.

INTRODUCTION

It has been estimated that more than 2×10^9 tons of livestock wastes are produced annually in the United States. Nearly 1.4×10^9 tons of these wastes are produced in cattle feedlot operations. Disposal of such large volumes of wastes represents a growing and critical problem with respect to environmental pollution (Miner and Smith, 1975), a financial problem to the livestock industry and a loss of large amounts of potentially usable nutrients. It has been estimated that the total collectible N in livestock wastes in the U.S.A. in 1972 was 2.2×10^9 Kg per annum which was roughly equivalent to the total N present in all the soybean crop produced in the U.S.A. that year (Yeck et al., 1975). Furthermore, the

amino acid composition of the protein in manure was reported to be comparable to that of soybean meal (Sloneker et al., 1973). Recycling feedlot wastes (FLW) as crude protein feed supplements is desirable since this will save disposal costs, reduce air and water pollution problems, lower cost of production of animal products and in turn reduce cost to the consumer, and finally increase the supply of available food protein in the world by sparing valuable nutritional resources such as cereal grains and soybeans for human consumption (Smith, 1973). Another advantage with FLW is its on-site concentration which allows continuous collection and processing.

A number of reviews have been published on recycling of FLW as livestock feeds (Anthony, 1971; Bhattacharya and Taylor, 1975; Federal Register, 1977; Ichhopani and Lodhi, 1976; Smith, 1973). Anaerobic lactic acid fermentation of whole manure was reported by Moore and Anthony (1970) but few details are available. Fermentative conversion of cattle FLW into single cell protein, using thermophilic actinomycetes, was reported by Bellamy (1974). Griffin et al (1974) reported that fresh FLW is an excellent substrate for the production of cellulolytic enzymes by *Trichoderma viride* and that the fermented waste was odor free and contained 24% less organic matter. Morrison et al. (1977) described procedures for the extraction and recovery of the protein constituents of FLW and subsequent fermentation of the extracted residues for the production of additional biomass. Although the practical

feasibility of the above processes was questioned, these studies none the less demonstrated the potential of FLW as a substrate for fermentative recycling processes.

Sloneker et al. (1973) fractionated cattle FLW into two fractions: a solid fibrous fraction and a liquid portion which contained nearly 70% of the total N originally in the waste. Approximately 45% of the total protein in this FLW liquid was shown to be of microbial origin (Morrison et al., 1977). The above fractionation followed by separate processing of the two fractions was considered essential for the fullest utilization of the waste (Rhodes and Orton, 1975). Weiner and Rhodes (1974a & b) reported on controlled aerobic fermentation of feedlot waste filtrate utilizing either natural flora or selected fungi and streptomycetes. These studies were primarily concerned with the reduction of biological oxygen demand, chemical oxygen demand, and odor problems associated with feedlot waste and secondarily with the production of microbial protein as a by-product. Weiner and Rhodes (1974b) further reported that addition of a readily fermentable carbohydrate source to FLW liquid significantly increased biomass yield of streptomycetes and various fungi. Rhodes and Orton (1975) developed a solid-substrate fermentation system in which FLW liquid was added to cracked corn at a ratio of 1:2 and the mixture (40% moisture content) was fermented aerobically at ∿35-37 C in a revolving cement mixer. Fermented corn had a crude protein (CP; Total N x 6.25) content of 10.8% as compared to 9.6% CP in unfermented

corn, or a marginal 12% improvement in CP due to fermentation under these conditions.

Reddy and Erdman (1977) recently described a batch fermentation process in which FLW filtrate, which is very low in fermentable carbohydrate, was augmented with carbohydrate-rich agricultural wastes such as cheese whey or starchy waste recovered from potato processing operations. Fermentations were conducted at 43 C and pH 5.5 for 24 h using either indigenous flora in FLW, mixed rumen flora or known pure cultures of lactobacilli as inoculum. Organic acids produced during the fermentation were continually neutralized with ammonia to produce ammonium salts of organic acids which were shown to be comparable to soybean meal as crude protein supplements for ruminants. The rationale for ammoniated organic acid fermentation of agricultural wastes was discussed at greater length in a recent review by Gerhardt and Reddy (1978). At the end of 24 h of fermentation, the product obtained had a CP content of about 80% on a dry basis. Erdman and Reddy (1978) optimized the above process and showed that at an optimal pH of 7.0-7.5, the total fermentation time could be reduced 3-4 fold (from 24 h to 6-8 h) which is considerably shorter than any other FLW fermentation process described to date.

The objective of the present investigation was to develop a continuous process for the fermentative conversion of FLW filtrate into a crude protein supplement for ruminant animals. A continuous process is more desirable than a batch process

because the former is more adaptable to instrumental control, is better intergrated into the preceding and subsequent processing operations, generally yields a more uniform product and the productivity of a continuous culture is usually higher than that for batch culture.

MATERIALS AND METHODS

Fresh ($\leq$ 1 d old) bovine FLW was collected from finishing steers fed a high silage ration containing 88% corn silage and 12% soy supplement (Fox and Cook, 1977).

Preparation of substrate for fermentation. FLW (16.5% total solids) was diluted 1:1 with tap water and mechanically agitated with a T-bar (20 x 80 x 1.3 cm diameter) until a homogenous slurry was obtained. The slurry was fractionated into solid and liquid portions by passing it through a pneumatic slurry separator (Gascoigne Gush and Dent, Agricultural Limited, Reading, Berkshire, England; pore size 3mm). The solid fibrous portion, and the filtrate fraction (FLWF, which contained most of the solubles and fine particulate matter), contained 81.4 and 18.6%, respectively, of the solids originally present in FLW. The filtrate fraction was stored in 208-liter mild steel barrels at 4 C until used ($\leq$ 7 d). Unless otherwise mentioned, FLWF, supplemented with cheese whey powder (CWP; Galloway West, Fond du Lac, WI) at a level of 5 g/100 ml ($\sim$3.5-3.9g lactose/100 ml), served as the fermentation substrate. The required amount of CWP was added to the FLWF, mixed thoroughly in a blender and the blended mixture, contained in a nonsterile carboy at

4 C, was used as the substrate.

Continuous fermentation procedures. A 28-liter fermentor (Model CMF-128S, New Brunswick Scientific, New Brunswick, NJ), equipped with automatic temperature and agitation controls was used throughout this study. A false bottom (constructed of 316 stainless steel, supported on sterile gravel and sealed with silicone sealer) was installed into the fermentor vessel to reduce the total operating capacity of the fermentor to 10 liters. Substrate feeding and product removal was accomplished by independently controlled peristallic pumps (Model T6S, Sigma motor, Middleport, NY) fitted with polyurethane tubing. A large bore glass tube fixed at the 10-liter level in the fermentor was connected to the product harvest pump which was operated at a rate slightly higher than the feeder pump to effectively remove the effluent into the product reservoir and thus maintain a constant volume in the fermentor.

To initiate continuous fermentation, the fermentor was charged with 10 liters of freshly prepared, unsterilized FLWF, supplemented with 50mg CWP/ml. No exogenous inoculum was added; indigenous microbial flora already present in the FLWF served as the inoculum. Unlike other ammoniated organic acid fermentations of wastes previously described (Reddy et al.,1976; Forney and Reddy, 1977; Keller and Gerhardt, 1975) no growth factor supplementation was required. Temperature was adjusted to and maintained at 43 C. The pH was maintained constant at 7.0 by the automatic

addition of ammonia as previously described (Reddy and Erdman, 1977). Ammonia addition also resulted in the production of ammonium salts of organic acids. The fermentation was allowed to proceed batchwise for 4 h, at which time the continuous feed and harvest were initiated. The fermentation was allowed to proceed continuously for at least 4 times the retention time (RT) and establish a steady state before a change was made to a new set of operating conditions. At no time during the fermentation were sterile or anaerobic precautions taken.

Analytical procedures. All samples were collected and stored in glass screw cap vials at -18 C until analyzed. At least two samples were collected for each set of experimental conditions. The interval between the two samples was equivalent to the RT at that particular steady state. The total nitrogen (TN) in various samples was determined by the micro-Kjeldhal procedure (Wall and Gherke, 1975). Total solids (TS) were determined gravimetrically by drying a 50-60 g sample at 50 C and reduced pressure to a constant weight. The supernatant obtained after centrifugation of the sample at 25,000 x g for 15 min was used for the following determinations. Lactose was estimated by the procedure of Dubois et al. (1956) as modified by Montgomery (1961). Ammonia nitrogen (AN) was determined by Nesslerization (Oser, 1965). Metabolic end products were analyzed by a Hewlett-Packard gas chromotograph (Model 5730A) equipped with a flame ionization detector and temperature programming.

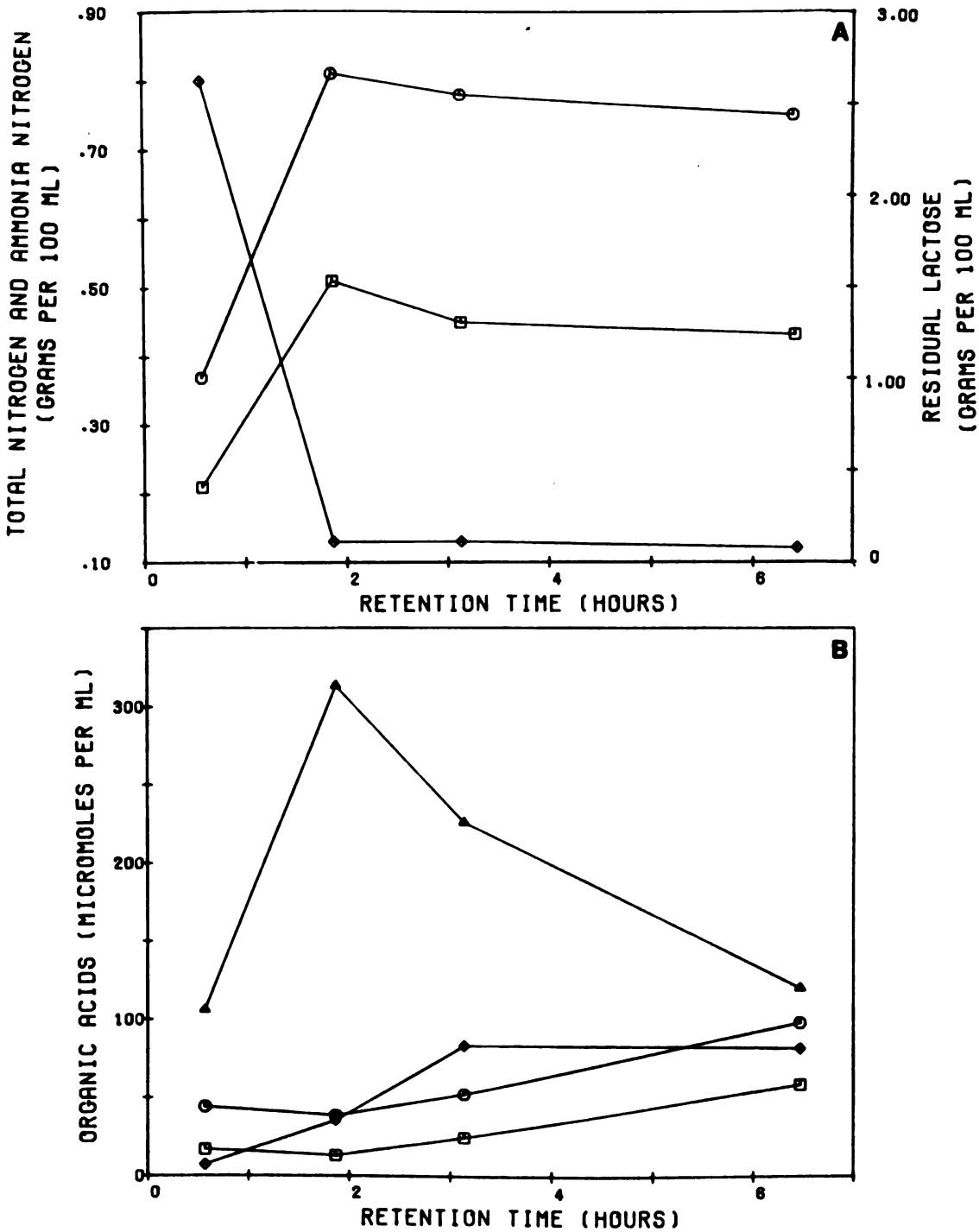
Volatile fatty acids (VFA) in a 2.0 µl sample were determined, using a column packing of 3% carbowax, 20M/0.5% H_3PO_4 on 60/80 carbopack B (Supelco, Bellefonte, PA) by the procedure of DiCorcia and Samperi (1974). Temperature of the column was increased from 100 C to 200 C at the rate of 4 C/min. Non-volatile organic acids (NVA) in the samples were methylated by the procedure of Holdeman and Moore (1972). A 2.0 µl sample was injected onto 10% SP-1000/1% H_3PO_4 on 100/120 Chromosorb W AW (Supelco). A column temperature of 90 C was employed initially which was gradually increased to 130 C at the rate of 4 C/min. The kinds and amounts of acids were determined by comparing their retention times and peak areas to retention times and peak areas of a standard concentration of known volatile and non-volative organic acids under the same experimental conditions.

RESULTS AND DISCUSSION

The effect of RT on concentrations of residual lactose, TN and AN is shown in Fig. 1A. The optimal RT for this study was arbitrarily defined as the shortest RT which gives the lowest concentration of residual lactose and the highest concentration of TN. At the optimal retention time of 1.9 h (dilution rate = 0.53 h^{-1}) the residual lactose concentration was 0.17 g/100 ml (equivalent to 95-96% utilization of the lactose added). At this RT 19.6 mg/ml/h of lactose was utilized by the microbial flora (Table 1). To the best of our knowledge, this is the highest rate of utilization of fermentable carbohydrate ever reported for a continuous fermentation

Figure 1A. The effect of retention time (RT) on concentrations (g/100 ml) of residual lactose (-◇-), ammonia nitrogen (-□-), and total nitrogen (-○-) during continuous fermentation of feedlot waste filtrate (FLWF), supplemented with cheese whey powder (5 g/100 ml), at 43 C and pH 7.0.

Figure 1B. Changes in the concentration of organic acids with changes in RT in the above fermentation. Symbols: lactic acid (-△-); acetic acid (-◇-); propionic acid (-○-); butyric acid (-□-).


A
TOTAL NITROGEN AND AMMONIA NITROGEN (GRAMS PER 100 ML)
RESIDUAL LACTOSE (GRAMS PER 100 ML)
.90
.70
.50
.30
.10
3.00
2.00
1.00
0
0
2
4
6
RETENTION TIME (HOURS)
B
ORGANIC ACIDS (MICROMOLES PER ML)
300
200
100
0
0
2
4
6
RETENTION TIME (HOURS)

process. In comparison, batch fermentation, continuous fermentation, and dialysis continuous fermentation of cheese whey gave lactose utilization rates of only 4.3 (Reddy et al., 1976), 1.6 (Keller and Gerhardt, 1975), and 7.5 mg/ml/h (Steiber et al., 1977) at retention times of 16,31, and 19 h, respectively. Furthermore, the RT of 1.9 h shown to be optimal for the continuous fermentation of FLWF (supplemented with CWP at 5% level) was 3-4 times shorter than the fermentation time of 6-8 h previously shown to be required for a batch fermentation of the same material (Erdman and Reddy, 1978). Shorter fermentation time would allow for smaller fermentor capacity, reduced capitol investment and lower production costs.

At a RT of 1.9 h, TN and AN concentrations in the product were maximal. The product had 70-75% CP on a dry basis. AN (in the form of ammonium salts of organic acids) constituted 65-70% of the total CP in the product. There was approximately 2.5-3.0 fold increase in CP content during the fermentation. Retention times greater than 1.9 h resulted in no detectable increase in the CP content of the product; in fact, there was a slight drop in TN and AN content in the product at higher retention times (Fig. 1A). At a RT of 0.6 h, the residual lactose content was unacceptably high (2.5%) and CP content was 2.2 times lower than the product obtained at a RT of 1.9 h.

Organic acid composition in the product was greatly influenced by changes in RT (Fig. 1B). At 1.9 h RT, lactate

was the predominant acid. The percent molar distribution of lactate:acetate:propionate:butyrate was 78:10:3:9 and the organic acids produced accounted for about 95-98% of the lactose utilized. At higher retention times there was a dramatic decrease in lactate concentration and increases in the concentrations of acetic, propionic and butyric acids. For example, at a RT of 6.5 h the percent molar distribution of lactate:acetate:propionate:butyrate was 34:27:16:23, respectively.

The rate of lactose utilization and the rates of increase in concentration of TN, AN and total organic acids were approximately inversely proportional to the RT (Table 1). Furthermore, rates of production of various organic acids varied considerably at different RTs. The residual lactose concentration at RTs of 1.9, 3.1 and 6.5 h was about the same (Fig. 1A) but the rates of lactose utilization at these RTs were noticeably different (Table 1). However, the percentage conversion of lactose carbon to organic acids was quite similar at the four RTs tested. It should be noted that at a RT of 0.6 h, although the residual lactose content was high ($\sim$2.5%), the rate of lactose utilization (22.5 mg/ml/h) and the rate of total organic acid production (221.9 μmoles/ml/h) were the highest. This is to be expected since the organisms which are retained at a RT of 0.6 h will be expected to have higher multiplication rates and in turn show greater rate of metabolism of the substrate compared to the microbial groups active at higher RTs.

Table 1. Effect of retention time on rate of utilization of lactose and on rates of increase in total nitrogen, ammonia nitrogen, and various organic acids during continuous fermentation of FLWF supplemented with cheese whey powder[a,b]

RT (h)	Rate of Lactose Utilization (mg/ml/h)	Rate of Increase TN (mg/ml/h)	Rate of Increase AN (mg/ml/h)	Rate of Production of Organic Acids (μmoles/ml/h) A	P	B	L	Total	Efficiency of Conversion of Lactose to Acids (%)
0.6	22.5	[d]	2.6	39.3	20.1	8.7	154.0	221.9	94.5
1.9	19.6	2.6	2.4	9.2	4.1	17.9	157.8	188.9	95.0
3.1	11.6	1.5	1.2	9.9	6.3	26.0	66.4	108.6	95.7
6.5	5.7	0.7	0.6	11.8	8.2	12.3	15.8	48.0	96.9

[a]Abbrevations used: RT = retention time; TN = total nitrogen; AN = ammonia nitrogen; A = acetic acid; P = propionic acid; B = butyric acid; L = lactic acid.

[b]Experimental conditions as described in Materials and Methods.

[c]These values were calculated based on the assumption that 1 mole of lactose yields 4 moles of acetate or propionate, or 2 moles of butyrate.

[d]Not determined.

The results (Fig. 1B and Table 1) suggest that bacterial populations in the FLWF undergo a continuum of changes at different RTs. Lactic acid producing bacteria appear to constitute the predominant population at a RT of 1.9 h. Probably these bacteria have higher specific growth rates and have a competitive edge over slower growing bacteria in the FLW which may be washed out at this particular RT. At higher RTs, lactate-producting bacteria become relatively less predominant, and propionate and butyrate-producing bacteria show considerable increase in numbers. Somewhat similar results were obtained during batch fermentation of FLWF, supplemented with 5% CWP, at 43 C and pH 5.5 (Erdman and Reddy, 1978). In the first 6-8 h of batch fermentation, lactic acid was the predominant product but during the succeeding 16-18 h of fermentation lactate concentration decreased by >90%, acetate concentration increased moderately or remained the same, and propionate and butyrate concentrations gradually increased.

The effect of supplementation of FLWF with different levels of cheese whey powder and the effect of changes in RT on residual lactose, TN and AN concentrations are shown in Table 2. Optimum RTs as determined by residual lactose concentrations $\leq$ 0.3% (equivalent to 92% utilization of lactose during fermentation), and maximum TN or AN in the product were 7.7 and 10.4 h for FLWF supplemented with CWP at 10 or 15% level, respectively. When FLWF was supplemented with 20% CWP, there was 1.85% residual lactose in the product even at a RT of 10.4 h. It should be noted, however, that even at

Table 2. Effect of the level of supplementation with cheese whey powder and retention time on concentrations of residual lactose, total nitrogen (TN) and ammonia nitrogen (AN) during fermentation of feedlot waste filtrate [a]

CWP[b] Conc. (mg/ml)	RT (h)	Residual[c] Lactose (mg/ml)	AN (mg/ml)	TN[c] (mg/ml)	Crude[c] Protein (% dry basis)	Rate of Lactose Utilization (mg/ml/h)	Rate of Increase TN (mg/ml/h)	Rate of Increase AN (mg/ml/h)	Efficiency[d] of conversion of Lactose to Acids (%)
100	3.0	28.0	6.6	9.3		15.5	1.95	1.95	
	7.7	3.0	7.2	10.2	58.9	8.7	0.83	0.83	69.1
	10.4	2.0	6.1	8.9		6.3	0.80	0.80	
150	3.0	34.0	8.7	10.8		25.7	2.16	2.42	
	7.7	23.0	8.6	10.0		13.3	0.84	0.92	
	10.4	2.3	9.1	11.5	48.9	9.8	0.70	0.73	69.5
200	3.0	107.0	7.5	10.0		13.5	1.74	2.01	
	7.7	60.0	10.5	12.8	45.5	11.6	1.08	1.27	
	10.4	18.0	9.3	12.6	44.7	12.4	0.77	0.86	71.8

[a] All fermentations were conducted at 43 C and pH 7.0.

[b] Cheese whey powder (CWP) contained approximately 66-70 g lactose per 100 g powder.

[c] Values given are for the product obtained at a specific retention time at a given level of supplementation with CWP.

[d] These values were calculated based on the assumption stated in footnote c in Table 1 and the amounts of acids produced as given in Table 3.

this RT the rate of lactose utilization (12.4 mg/ml/h) was very high as compared to previously reported values (Steiber et al., 1978). The results also showed that increased level of CWP supplementation does not result in a stoichiometric increase in TN in the product. For example, TN in the product was 0.81% when FLWF was supplemented with 5% CWP (Fig. 1A) and only 1.15 (as compared to the expected value of 1.8%) when supplemented with CWP at the 15% level (Table 2). In fact, CP (dry basis) in the product actually went down at increasing levels of CWP supplementation (Table 2). These results indicate that there is no real advantage in supplementing FLWF with CWP at levels higher than 5%. At 5% level of CWP supplementation, the efficiency of conversion of substrate to organic acids was $\geq$ 95% while at 10 or 15% level of CWP supplementation, the corresponding efficiency was only about 70%. Recent results by Erdman et al. (unpublished data), in agreement with the above results, showed that during batch fermentation of FLWF supplemented with CWP at 5,10,15 or 20% level, fermentation efficiency decreased at higher levels of supplementation. Furthermore, in the continuous process, TN and AN values decreased by 26.6 and 36.6%, respectively, at 10% CWP supplementation, and by 32.7 and 51.9% at 15% CWP supplementation as compared to the batch fermentation of FLWF supplemented with corresponding levels of CWP (Erdman et al., unpublished data).

The results showed that rates of production of various organic acids during the fermentation are greatly influenced

Table 3. The effect of level of supplementation with cheese whey powder (CWP) and retention time on concentration of various organic acids produced during the fermentation of feedlot waste filtrate[a]

CWP Conc. (mg/ml)	RT (h)	Conc. of Organic Acids[b] (μmoles/ml)				Rate of Production of Organic Acids (μmoles/ml/h)			
		A	P	B	L	A	P	B	L
100	3.0	95.1	59.1	40.4	261.8	22.0	18.3	12.3	79.2
	7.7	112.8	93.0	140.4	153.9	10.8	11.5	17.8	16.6
	10.4	123.3	32.4	200.1	58.8	9.0	2.7	18.8	3.2
150	3.0	105.8	62.0	47.8	326.7	23.0	19.5	15.6	93.0
	7.7	120.1	132.2	109.2	225.8	10.8	16.7	14.0	22.9
	10.4	123.8	32.8	277.6	191.4	8.3	2.8	26.6	13.6
200	3.0	80.1	42.9	41.9	246.7	22.2	13.6	13.7	61.7
	7.7	114.4	111.1	109.8	243.5	13.0	14.1	14.1	23.5
	10.4	153.7	143.6	246.6	212.5	13.4	13.6	23.6	14.4

[a]All fermentations were conducted at 43 C and pH 7.0. Other experimental conditions were as given in Table 2. Abbreviations as given in the footnote for Table 1.

[b]Values given are for the product obtained at a specific retention time at a given level of supplementation with CWP.

by the RT and level of supplementation with CWP (Table 3). When FLWF was supplemented with CWP at 10 or 15% level, lactate concentration in the product as well as the rate of production of lactic acid decreased sharply, and acetate and butyrate concentrations gradually increased with increasing RT (Table 3). Up to a RT of 7.7 h, propionate concentration rose gradually, but at 10.4 h RT its concentration decreased by 25 to 35% suggesting the possible presence of propionate-utilizing microbial population under these conditions. The results (Tables 2 and 3) also showed that the amounts of CWP added to the FLWF, and the RT exert a marked influence on the relative molar proportions of lactate:acetate:propionate: butyrate. These values were 31:20:5:44, 31:23:18:28 and 78: 10:3:9, respectively, for FLWF supplemented with 15,10 or 5% CWP.

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SECTION 4 (ARTICLE 4)

PRODUCTION OF NITROGENOUS FEED SUPPLEMENTS FOR RUMINANTS BY BATCH FERMENTATION OF CHEESE WHEY-SUPPLEMENTED POULTRY WASTE, SWINE WASTE, AND CATTLE FEEDLOT WASTE FILTRATES AND MIXTURES OF THESE WASTES

By

M. D. Erdman and C. A. Reddy

SUMMARY

Fermentative conversion of poultry and swine waste filtrates (SWF and PWF) into nitrogenous feed supplements for ruminants has been studied. Wastes were fermented, with or without cheese whey (CW) supplementation, either individually or in 1:1 combination with each other at 43 C and pH 7.0, utilizing indigenous flora as the inoculum. The pH was maintained constant by the automatic addition of ammonia. Fermentation of unsupplemented wastes resulted in little increase in total nitrogen (TN), but ammonia nitrogen (AN) increased 8.3 fold in fermented PWF. Fermentation of CW-supplemented PWF, SWF and FLWF resulted in products containing 62, 47.4 and 72% crude protein (CP), respectively, on a dry basis; AN accounted for 63,53 and 66% of the CP, respectively. Lactate (73%) and acetate (20%) were the major acids in CW-PWF fermentation, whereas lactate (30%), acetate (29%), and propionate (26%) were the predominant acids in CW-SWF fermentation. Fermentation of CW-supplemented PWF + SWF and PWF + cattle feedlot waste filtrate (FLWF) resulted in products containing 51.6 and 56.7% CP, respectively; AN accounted for 81 and 61% of the CP, respectively. Lactate and acetate accounted for 69 and 21%, respectively, of the total acids produced in CW-supplemented PWF + FLWF fermentation, whereas in corresponding PWF + SWF fermentation, lactate, acetate and propionate were predominant and accounted for 54.5,24.5 and 15.7% of the total acids produced, respectively. In all fermentations, utilization of the carbohydrate in the substrate was > 90% within

8 hr. The results indicate that ammoniated organic acid fermentation is a rapid and efficient means of recycling livestock wastes as potential nitrogenous feed supplements to ruminants.

(KEY WORDS: Poultry Waste; Swine Waste; Feedlot Waste; Waste Recycling; Feed From Wastes; Waste Fermentation)

INTRODUCTION

Disposal of the large volumes (2×10^9 tons) of livestock wastes produced annually in the USA represents a serious environmental pollution problem and an economic and nutrient loss as well (Blair and Knight, 1973a; Loehr, 1968; Miner and Smith, 1975; Sloneker et al.,1973; Syrett, 1977; Yeck et al., 1975). Recycling livestock wastes as animal feeds, after appropriate processing, is an attractive alternative to disposal of wastes and a number of reviews have been published on this subject (Anthony, 1971; Smith, 1973; Bhattacharya and Taylor, 1975; Blair and Knight, 1973b; Moellers and Vetter, 1974; Calvert, 1974, 1979; Ichhponani and Lodhi, 1976; Anonymous, 1977). Ammoniated organic acid fermentation of agricultural wastes (Gerhardt and Reddy, 1978) into nitrogenous feed supplements appears to be a novel and efficient solution to the problem of agroindustrial wastes. This approach has successfully been used for recycling cheese whey as crude protein feed supplement for ruminants (Reddy et al., 1976; Crickenberger et al., 1977; Huber et al., 1976; Henderson et al., 1974a,b,1975; Steiber and Gerhardt, 1978). This

particular approach appears useful for fermentative recycling of cattle feedlot waste filtrate (FLWF) also (Reddy and Erdman, 1977; Erdman and Reddy, 1978,1979). In the latter process (Reddy and Erdman, 1977), FLWF, supplemented with a complementary carbohydrate-rich waste as exemplified by cheese whey, was fermented under controlled conditions and the organic acids produced were neutralized with ammonia to produce ammonium salts of organic acids which were shown to be excellent sources of dietary nitrogen and energy for ruminants (Allen and Henderson, 1972; Henderson, 1974a,b, 1975; Atwall et al., 1974; Hungate, 1966; Dutrow et al., 1974; Howes, 1972). Based on the results of the FLWF process above, it appeared feasible to develop simple and efficient procedures for the production of ruminant feed supplements by fermentation of poultry, swine and cattle feedlot waste filtrates and mixtures of these wastes. These results are presented in this paper.

MATERIALS AND METHODS

Fresh ($\leq$ 1 day old) poultry waste (PW; from 20 to 24 month old Delkalb leghorn hens) was collected from concrete floors in a 5000 head, caged house. Hens were fed a typical layer ration (Flegal et al., 1975).

Fresh swine waste (SW) was collected from the surface of concrete slotted pens containing feeder pigs (Hampshire and Yorkshire, 59 kg) which were fed a corn-soybean ration fortified with lysine (Miller, 1975).

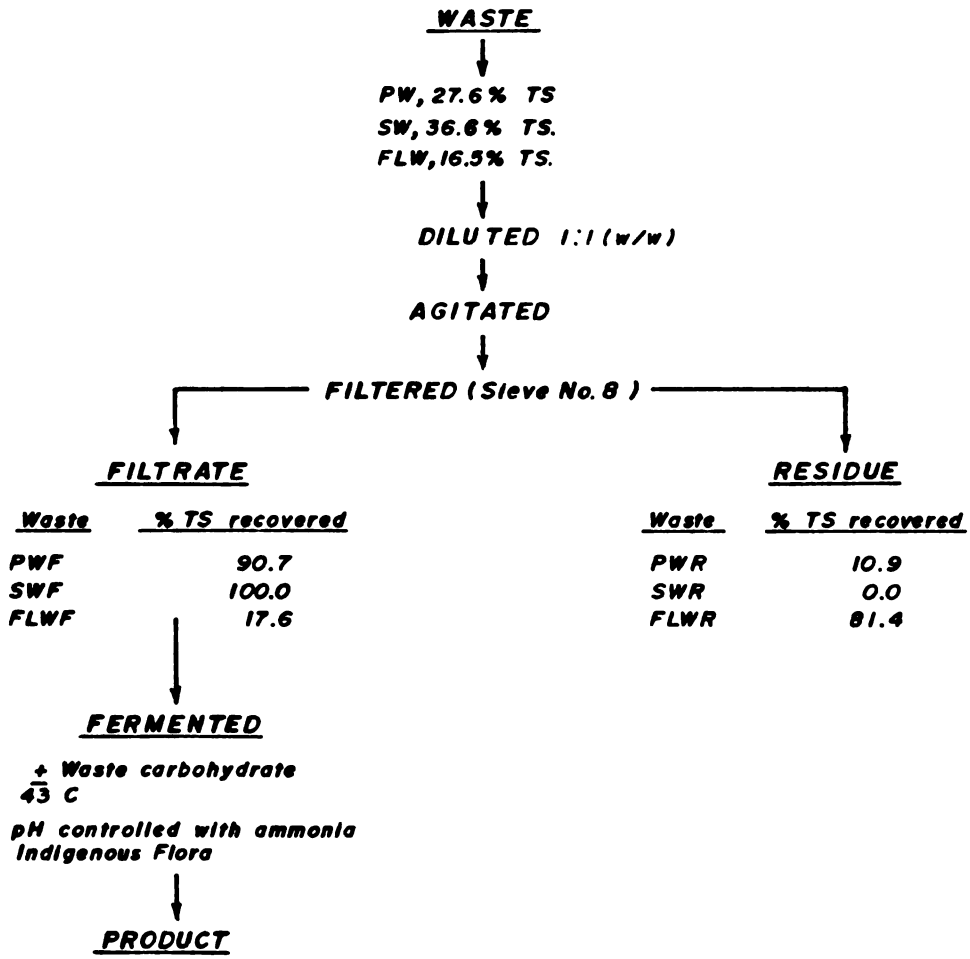
Fresh feedlot waste (FLW) was collected from uncovered, concrete floors containing steers (Hereford x Angus,

317 to 362 kg) fed a corn-corn silage ration (Fox and Cook, 1977).

The fractionation and fermentation scheme used for PW, SW and FLW is presented in Figure 1. PW, SW and FLW contained 27.6,35.6 and 16.5% total solids (TS), respectively. Each type of waste was collected and individually processed. The waste was diluted 1:1 (w/w) with tap water and mechanically agitated until a homogenous slurry was obtained. The slurry was then fractionated into filtrate and residue fractions by passing through a sieve No. 8. After fractionation, 90.7% of the TS in PW and 100% of the TS originally present in SW were present in the respective filtrates. In contrast, FLWF accounted for only 17.6% of the original TS content in FLW. The filtrate fraction was used for fermentation in all cases. For fermentations of mixtures of animal waste filtrates, equal volumes of the appropriate filtrates were blended and this mixture was used for fermentation. In most fermentations, the individual filtrate or 1:1 mixture of two differnet filtrates were supplemented with cheese whey powder (CWP, Michigan Milk Producers, Ovid, MI) at 5 g/100 ml.

A 28-liter fermentor Model CMF-128S, New Brunswick Scientific, New Brunswick, NJ), equipped with automatic temperature, agitation and pH controls, was used throughout this study. The fermentor was operated at the 20-liter level, temperature was maintained at 43 C and pH controlled at 7.0 by the automatic addition of ammonia. All other procedures were as previously described (Reddy and Erdman, 1977).

Figure 1. Schematic depiction of the fractionation and fermentation procedures used for poultry waste (PW), swine waste (SW) and feedlot waste (FLW). PWF, SWF and FLWF represent poultry waste filtrate, swine waste filtrate and cattle feedlot waste filtrate, respectively. PWR, SWR and FLWR represent poultry waste residue, swine waste residue and cattle feedlot waste residue, respectively. TS represents total solids.


WASTE
PW, 27.6% TS
SW, 36.6% TS.
FLW, 16.5% TS.
DILUTED 1:1 (w/w)
AGITATED
FILTERED (Sieve No. 8)
FILTRATE
Waste
% TS recovered
PWF
90.7
SWF
100.0
FLWF
17.6
RESIDUE
Waste
% TS recovered
PWR
10.9
SWR
0.0
FLWR
81.4
FERMENTED
+ Waste carbohydrate
43 C
pH controlled with ammonia
Indigenous Flora
PRODUCT

Samples were collected periodically throughout the fermentation and immediately stored in glass screw-capped vials at -18 C until analyzed. Samples were analyzed for total nitrogen (TN), ammonia nitrogen (AN), organic acids, lactose and TS as previously described (Erdman and Reddy, 1978).

RESULTS

Unsupplemented Fermentations. The composition of unsupplemented PWF,SWF and FLWF before and after fermentation are presented in Table 1. The CP content was low in all three types of wastes prior to fermentation. However, unfermented PWF (6.8% CP) and SWF (4.5% CP) contained 4.2 and 2.8 fold more CP, respectively, than that in FLWF (1.6%). Fermentation of unsupplemented wastes resulted in no apparent increase in CP content with any of the wastes examined. However, AN concentration increased rather dramatically during fermentation of unsupplemented PWF and SWF (8.3 and 2.5 fold increase in AN, respectively) and represented 66.7 and 74.8% of the TN present. Organic acids were qualitatively and quantitatively changed during fermentations of PWF and SWF. For example, during fermentation of PWF, the concentration of acetic acid increased 75%, propionic and butyric acids decreased 34 and 19%, respectively, and lactic acid was not detected at the end of 24 hr. In contrast, little changes in organic acids occurred during fermentation of FLWF.

CWP-Supplemented Fermentations. The effect of supplementation with a readily fermentable complimentary waste

Table 1. Composition of unsupplemented poultry waste filtrate (PWF), swine waste filtrate (SWF) and feedlot waste filtrate (FLWF) before and after fermentation[a]

Component	PWF		SWF		FLWF	
	Time (hr)		Time (hr)		Time (hr)	
	0	24	0	24	0	24
Total nitrogen[b]	1.09	1.11	.72	.72	.26	.27
Ammonia nitrogen[c]	.10	.83	.19	.48	.02	.04
Lactose[c]	.03	.03	.2	.2	.1	.1
Total acids[d]	121.1	141.0	35.8	91.0	28.8	34.6
Acetic	61.6	107.7	18.7	50.7	22.0	27.6
Propionic	25.2	16.6	10.9	27.1	3.7	5.4
Butyric	20.6	16.7	6.2	13.2	.4	1.3
Lactic	13.7	.0	.0	.0	2.7	.3

[a]Fermentations were conducted as described in Methods.

[b]Expressed in g/100 g of filtrate.

[c]Expressed in g/100 ml of clarified filtrate, see Methods.

[d]Expressed in μmoles/ml of clarified filtrate, see Methods.

carbohydrate, as exemplified by CWP, on fermentation of PWF, SWF and FLWF was examined and the results are shown in Fig. 2 (A, B and C). As shown in Fig. 2C, whey lactose was utilized rapidly during fermentation of all the three wastes and > 90% of the added whey lactose was utilized within 6 hr. TN (Fig. 2A) and AN (Fig. 2B) contents increased rapidly through 6 to 8 hr of fermentation and remained relatively constant thereafter, except that AN content of PWF increased throughout 24 hr. TN concentrations in FLWF, SWF and PWF were 0.87, 1.22 and 1.49 g/100 g after 6 hr of fermentation which represented 164,65 and 37% increase in TN, respectively. AN accounted for 55,66 and 78% of the TN, respectively, in SWF, FLWF and PWF at the end of 24 hr fermentation.

Changes in the concentration of various organic acids at different times during the fermentation of supplemented PWF and SWF are presented in Fig. 3A and 3B. Total organic acids concentration was 632 μmoles/ml, after 8 hr of fermentation in supplemented PWF. Lactic and acetic were the predominant acids (Fig. 3A) and accounted for 73 and 20%, respectively, of the total organic acids produced. Minor amounts of propionic and butyric and trace amounts of isobutyric, fumaric and succinic acids were also observed during the fermentation of PWF.

During fermentation of supplemented SWF (Fig. 3B), lactic acid concentration increased rapidly during the first 2 hr, then remained relatively constant for 4 hr and thereafter decreased rather dramatically. Acetic and propionic

Figure 2. Rates of increase in the concentration of total nitrogen (A), ammonia nitrogen (B) and lactose utilization (C) with time during fermentation of poultry waste filtrate (-◇-), swine waste filtrate (-□-) and feedlot waste filtrate (-⬡-) supplemented with cheese whey powder (5 g/100 ml).

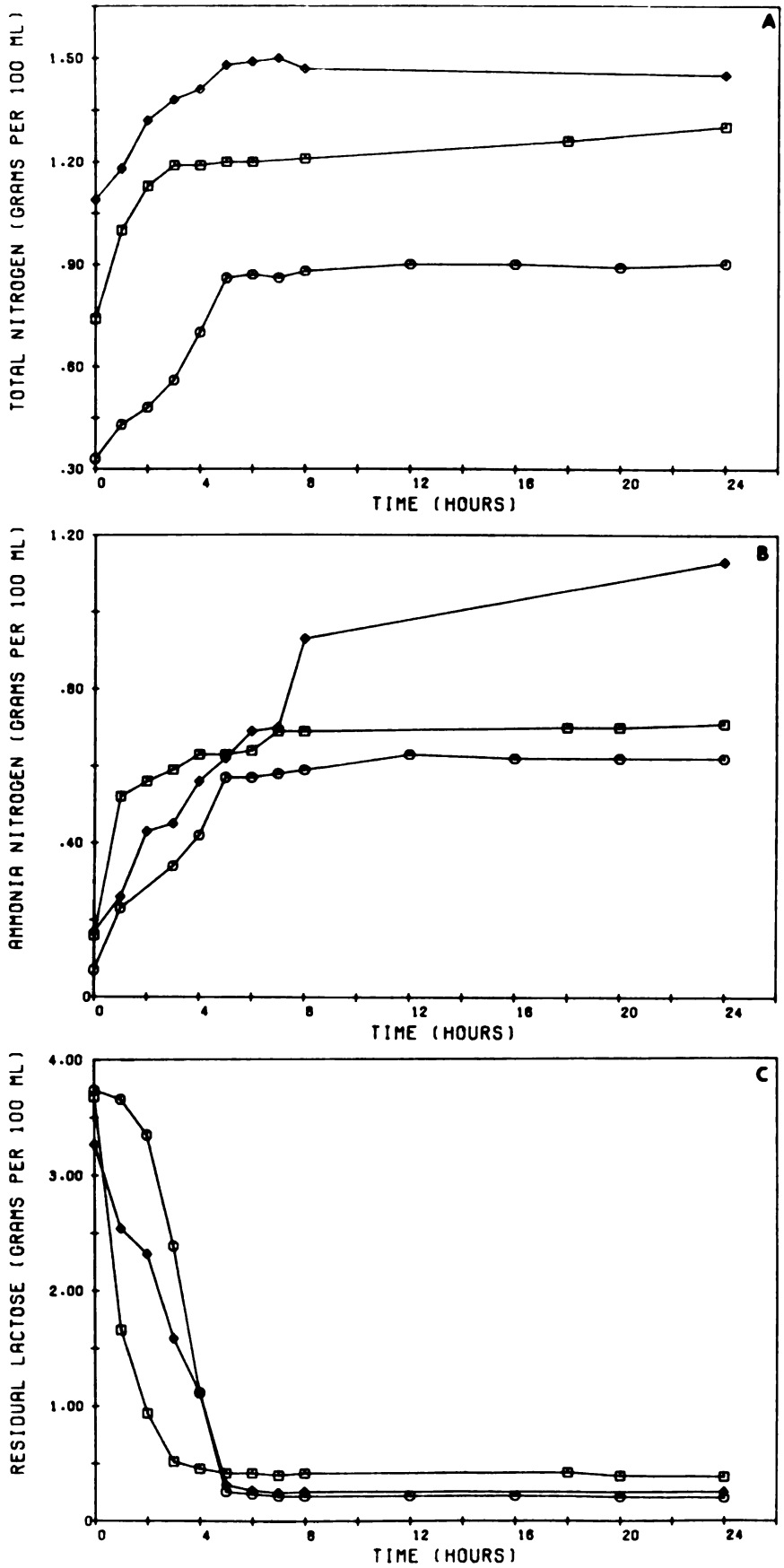
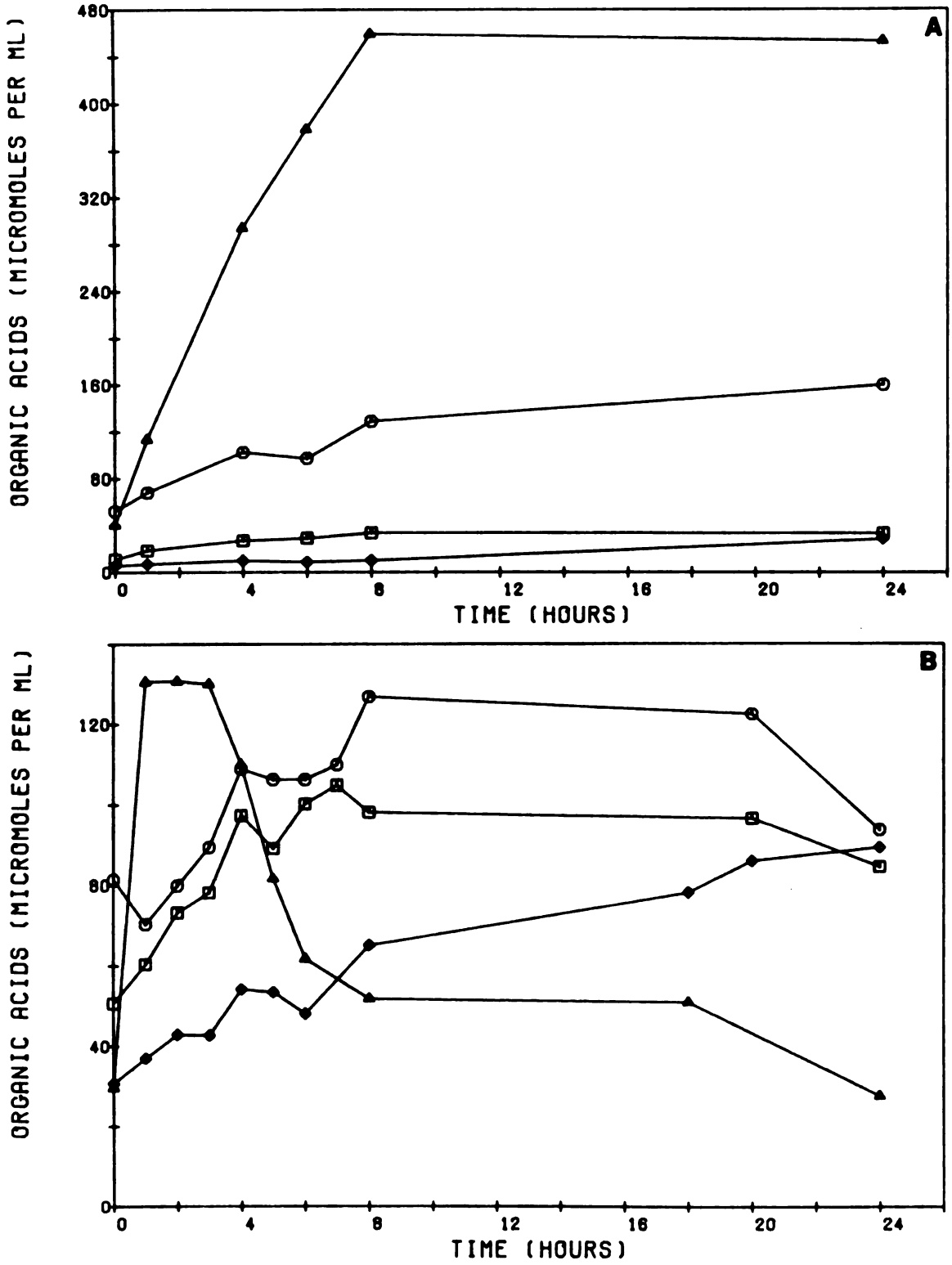

A
TOTAL NITROGEN (GRAMS PER 100 ML)
1.50
1.20
.90
.60
.30
0
4
8
12
16
20
24
TIME (HOURS)
B
AMMONIA NITROGEN (GRAMS PER 100 ML)
1.20
.80
.40
0
TIME (HOURS)
C
RESIDUAL LACTOSE (GRAMS PER 100 ML)
4.00
3.00
2.00
1.00
0
TIME (HOURS)

Figure 3. Changes in concentrations of acetic (-○-); propionic (-□-); butyric (-◇-); and lactic (-△-) acids with time during fermentation of poultry waste filtrate (A) or swine waste filtrate (B). Each substrate was supplemented with cheese whey powder (5 g/100 ml).


A
ORGANIC ACIDS (MICROMOLES PER ML)
480
400
320
240
160
80
0
0
4
8
12
16
20
24
TIME (HOURS)
B
ORGANIC ACIDS (MICROMOLES PER ML)
120
80
40
0
0
4
8
12
16
20
24
TIME (HOURS)

acid concentrations generally increased through 8 hr, remained relatively constant through 17-18 hr, and then gradually decreased through 24 hr. In contrast, butyric acid concentration increased throughout 24 hr of fermentation. The concentrations of total organic acids were 192,371 and 295 μmoles/ml at 0,4 and 24 hr of fermentation. Lactic, acetic and propionic acids represented 30,29 and 26%, respectively, of the total acids at the end of 4 hr.

The concentration of lactic and acetic acids rapidly increased and reached a peak at 6 hr during the fermentation of supplemented FLWF (results not shown). Lactic and acetic acids accounted for 72 and 26%, respectively, of the total acids produced at this time. Between 6 to 24 hr, lactic acid concentration decreased dramatically, propionic and butyric concentrations increased and acetic acid concentration remained relatively constant. The percent distribution of lactic, acetic, propionic and butyric acids at the end of 16 and 24 hr of fermentation were 49,36 11 and 4%, and 24,34,14 and 28%, respectively.

CWP-Supplemented Mixed Fermentations. The high TN content of PWF, most of which is known to occur as urea and uric acid (Bhattacharya and Taylor, 1975), and the observation that 66% of the TN is converted to AN during the fermentation (see Table 1) prompted us to investigate the feasibility of fermenting PWF mixed with FLWF or SWF and to examine if such a mixed fermentation will lower the requirement for exogenous ammonia or might otherwise favorably affect the

fermentation. The data on changes in TN, AN, whey lactose and organic acids during such mixed fermentations are shown in Table 2.

Fermentation of 1:1 mixture of PWF + FLWF, supplemented with CWP for 8 hr, resulted in a product containing 6.3% CP on a wet basis or 56.7% on a dry basis. The AN accounted for 78% of the CP present at this time. Most of the added lactose (92%) was metabolized within 8 hr. TN and AN increased 73 and 627%, respectively, during the fermentation. The total organic acids concentration was 486 μmoles/ml at 8 hr. Lactic and acetic acids were the predominant products and accounted for 69 and 21% of the total acids present at this time. Propionic (8%) and butyric (2%) acids were only minor products. Continuing this fermentation for 24 hr resulted in a slight decrease in TN and a corresponding increase in AN and a 38% decrease in the concentration of organic acids. The relative proportion of various acids also changed at the end of 24 hr: acetic > butyric > lactic > propionic.These results clearly indicated that it is desirable to terminate the fermentation at the end of 8 hr and that fermenting PWF + FLWF combination results in a product higher in TN and AN than that obtained with supplemented FLWF, but lower in TN and AN as compared to the fermentation product from supplemented PWF. Furthermore, the amount of exogenous ammonia consumed during the above mixed fermentation was 14% less than that added during the fermentation of supplemented FLWF (data not shown), indicating that PWF and FLWF are complimentary to

Table 2. Changes in composition during fermentation of cheese whey supplemented PWF + FLWF and PWF + SWF[a]

Mixture	Time	TN[b]	AN[b]	Lactose[b]	Total Acids[b]	Percent			
						A	P	B	L
PWF + FLWF	0.0	.59	.11	2.90	83.9	52.3	13.6	12.4	21.7
	4.0	.91	.62	1.36	315.2	20.7	9.3	2.0	68.0
	8.0	1.02	.80	.24	486.5	20.7	8.3	1.9	69.1
	24.0	1.01	.82	.14	302.7	30.6	15.4	29.7	24.3
PWF + SWF	0.0	.78	.12	3.34	127.9	45.9	18.7	13.7	21.7
	4.0	1.22	.59	1.02	406.1	22.9	15.6	5.6	55.9
	8.0	1.26	.77	.31	470.0	24.5	15.7	5.3	54.5
	24.0	1.26	.82	.27	307.1	39.0	23.6	25.2	12.2

[a]Fermentation conditions described in Methods. Abbreviations: TN, total nitrogen; AN, ammonia nitrogen; A, acetic; P, propionic; B, butyric; L, lactic.

[b]Expressed in g/100 g fermented filtrate.

each other and where feasible it is advantageous to ferment a combination of these two wastes.

Fermentation of supplemented PWF + SWF for 8 hr resulted in a product containing 7.9% CP on a wet basis or 51.6% on a dry basis; ammonia nitrogen accounted for 61% of the CP. TN and AN increased 62 and 542%, respectively, and 90.7% of the added lactose was metabolized during the fermentation. The total organic acids concentration was 470 μmoles/ml at 8 hr. Lactic and acetic acids were again predominant, accounting for nearly 80% of the total acids. The relative proportions of lactic, acetic, propionic and butyric acids were 55:24:16:5, respectively. Continuing this fermentation for 24 hr resulted in no further increase in TN content, and only a slight increase in AN, indicating that it might be preferable to terminate the fermentation at 8 hr. Total organic acids concentration decreased by about 35% at the end of 24 hr of fermentation and the relative proportion of various acids also changed dramatically at this time: acetic > butyric > propionic > lactic. It should also be noted that fermenting PWF + SWF combination supplemented with CWP resulted in a product higher in TN and total organic acids concentrations than that obtained with CWP-supplemented FLWF, but similar to the fermentation product obtained from CWP-supplemented SWF. Interestingly, the amount of exogenous ammonia added during the fermentation of PWF + SWF was the same as that added during the fermentation of supplemented SWF. Thus, there appears to be no real advantage with

supplemented PWF + SWF mixed fermentation as compared to supplemented SWF fermentation.

DISCUSSION

The results show that ammoniated organic acid fermentation of PWF, SWF, or mixtures of PWF and FLWF, or PWF and SWF, supplemented with CWP at the 5% level, can be conducted efficiently batchwise at pH 7.0 and 43 C using indigenous microbial flora as the inoculum. Under these conditions, fermentation of the added substrate is essentially complete in 4-8 hr. The product obtained in each case is superior to the starting material in CP and total organic acids content. The products of different fermentations contained 47-72% CP on a dry basis. The length of these fermentations are several fold shorter than the FLWF fermentation processes previously described (Rhodes and orton, 1975; Weiner, 1977a, b; Reddy and Erdman, 1977; Morrison et al., 1977; Weiner and Rhodes, 1974; Jackson et al.,1970) but comparable to that described for an optimized batch FLW process described by Erdman and Reddy (1978). Furthermore, by the processes described here, 100% of the solids in SW, about 91% of the solids originally present in PW, but only 18% of the solids in FLWF, can be recycled as a potentially valuable ruminant feed. Previous research on fermentative conversion of PW has been meager. Jackson et al. (1970) reported that under aerobic conditions, PW supplemented with 0.1% peptone water supports bacterial growth to a level of 1.6×10^9 bacteria

per ml. Vuori and Nasi (1977) reported an increase in the total amino acids concentration from 76 g to 136 g/kg during fermentation of PW inoculated with a uric acid-degrading yeast strain. In contrast, by the process proposed in this study, the fermentation time is much shorter and CP yields are several fold higher. Also two complimentary types of wastes can be recycled together and, in addition, several other advantages (see below) also result.

There have been a number of studies in the past few years on fermentative conversion of SW into animal feeds. Henry et al.,(1976) grew *Candida ingens*, a pellicle-forming yeast on effluents from a commercial swine farm and reported yields of 1.4 g microbial protein per liter of medium. Laboratory and pilot plant scale solid substrate fermentation of corn supplemented with SW was investigated by Weiner (1977a,b). Garret and Allen (1976) and Wilson and Houghton (1974,1977) grew bacteria and algae in SW primarily to reduce biological oxygen demand and chemical oxygen demand, but at the same time producing an algal crop as a by-product for possible use as an animal feed. Similarly, Chung et al. (1978) grew Spirulina on effluents from a SW methane generator. Yield of algae was 5 g/M^2/day and the algae contained 60% protein.

Previous studies have shown that the addition of readily fermentable carbon sources to livestock wastes increases the rate of fermentation and the amount of organic acids and/or single cell proteins produced (Weiner and

Rhodes, 1974; Vuori and Nasi, 1977; Reddy and Erdman, 1977; Weiner, 1977a,b). In agreement with these earlier studies, fermentation of unsupplemented wastes results in no appreciable increase in CP content, but supplementation with CWP greatly increased the CP content during the fermentation of SWF, PWF, PWF + SWF and PWF + FLWF.

Since CP content in rations is generally the most expensive component, any process aimed at recycling a waste should conserve as much of the utilizable nitrogen in the waste as possible. Although dried poultry waste (DPW) has been extensively studied and was shown to be a useful feed supplement to ruminants as well as other livestock species (Bhattacharya and Taylor, 1975; Flegal et al., 1975), it has been reported that as much as 15% of the TN in the fresh PW may be lost by volatilization during the drying process. In contrast, during PWF fermentation by the indigenous flora as described here, practically all the nitrogen in the initial material is conserved. Furthermore, the results suggest that urea and uric acids, which are the main nitrogenous components in fresh PW, are being converted to a large extent to AN by the microbiota in PWF. AN is efficiently utilized by rumen microbes for synthesis of microbial cell protein which is, in turn, the main source of protein to the ruminant.

Maximum amounts of organic acids are produced within 8 hr during the fermentation of CWP-supplemented SWF and PWF. This is followed by extensive qualitative and quantitative changes in these acids between 8 to 24 hr indicating the

occurrence of dynamic microbial population changes. For example, in fermenting PWF supplemented with CWP, lactic and acetic acids reach a peak in 8 hr and remain relatively constant through 24 hr. In contrast, in FLWF fermentation, lactic and acetic acids are predominant at the end of 6-8 hr; however, this is followed by a dramatic decline in lactic acid concentration and a corresponding rise in propionic and butyric acids between 8-24 hr, while acetic acid level remains essentially the same (Erdman and Reddy, 1978;1979). SWF fermentation appears different than FLWF and PWF fermentations in that lactic, acetic, propionic and butyric acids were all predominant in the first 2-6 hr, but after 6 hr lactic acid is metabolized rapidly and butyric acid concentration increases. Decreases in concentration of acetic, propionic and lactic acids between 20-24 hr may represent secondary metabolism of these acids. These results show that different microbial populations following different types of metabolism are active during fermentation of different wastes and that there is no real advantage in extending the fermentation beyond 8 hr. In fact, this may be a distinct disadvantage because as much as 35% of the organic acids undergo secondary metabolism between 8-24 hr. Therefore, terminating the fermentation at 8 hr may conserve the energy value of the product.

An important concern in fermentative recycling of agricultural wastes as nitrogenous feeds is the potential harm to the consuming animals by the pathogenic microbes and/or toxic residues that may be present in the processed

wastes. These aspects have been discussed in a recent paper by Fontenot and Webb (1975). In the study presented here, fermentation samples were routinely submitted to the Veterinary Clinical Diagnostic Microbiology Laboratory at Michigan State University. All samples were reported to be negative for obvious aerobic, faculative or anaerobic pathogenic bacteria. When formaldehyde was added to the fermentation product to give a final concentration of 1% to stop the microbial activity, and the product was cultured 48 hr after addition of formaldehyde, no viable microorganisms could be recovered on commonly used nonselective bacterial isolation media. Therefore, stabilization of the product by adding formaldehyde or by pasteurization is recommended before feeding these products to animals unless further investigations show that this precaution is unwarranted. Nutritional studies with yearling steers using formaldehyde-treated, supplemented FLWF product are currently in progress.

In conclusion, the results of this work indicate that ammoniated organic acid fermentation of PWF, SWF, FLWF, and mixed wastes is an effective means of converting these wastes into potentially useful nitrogenous feed supplements for ruminants. The proposed process offers a number of advantages over the previously described processes which include the following: (a) the CP yields on a dry basis are higher than any previously described waste recycling processes (Morrison et al., 1977; Rhodes and Orton, 1975; Weiner, 19771,b); (b) fermentation is complete within 4-8 hr, which

is shorter than that for any other livestock waste fermentation process described to date; (c) the process is anaerobic and, therefore, eliminates the high aeration costs associated with aerobic fermentation. Furthermore, most of the substrate carbon is conserved as organic acids in this process whereas in aerobic fermentation, much of the substrate carbon is lost as CO_2; (d) the process proposed is non-aseptic and saves sterilization costs; (e) there is no need to add separate inoculum or a source(s) of growth factors and, thus, further economy in operation costs is achieved; (f) there is no need to recover the product as in single cell protein processes; and (g) the process is simple to operate and the equipment needs are minimal.

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