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# A Novel Fermentation Process for Calcium Magnesium Acetate (CMA) Production from Cheese Whey

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## ABSTRACT

A novel, anaerobic fermentation process is developed to produce calcium magnesium acetate (CMA) from cheese whey. CMA can be used as a noncorrosive road deicer. It poses no environmental threats and has many advantages over road salt. A coculture consisting of homolactic and homoacetic bacteria was used to convert whey lactose to lactate and then to acetate in a continuous, immobilized cell bioreactor. The acetate yield from lactose was ~95% (wt/wt) in this homofermentative process. The acetic acid produced from fermentation was recovered in a concentrated CMA solution by using an energy-efficient extraction process. The development of a novel, extractive fermentation process to reduce product inhibition and to further increase reactor productivity is also discussed.

**Index Entries:** Calcium magnesium acetate; whey; fermentation; extraction; acetic acid.

## INTRODUCTION

Whey is a byproduct from the manufacture of cheese and casein. It contains about 5% lactose, 1% protein, 1% salts, and 0.1–0.8% lactic acid. The biological oxygen demand (BOD) of whey is high, ~40,000 mg/L. The annual production of cheese whey in the United States has continuously increased to about 57 billion pounds (26 million metric tons) in 1988. Currently, only ~50% of whey produced in the United States is used in

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human food and animal feed. The rest of the surplus whey must find a new use or be treated as a pollutant because of the high BOD of whey. With continuous increases in milk and cheese production in the United States and throughout the world, disposing of surplus cheese whey is one of the most critical problems facing the dairy industry.

Although whey protein generally can be recovered from whey via ultrafiltration, the remaining lactose stream, whey permeate, represents a major disposal problem. The utilization of whey lactose as a fermentation feedstock has been of great interest to the dairy industry (*1*). A wide range of products can be obtained from whey fermentations, including single-cell protein, methane, alcohols (ethanol, butanol), organic acids (lactic, acetic, propionic, citric), vitamins, and biopolymers (xanthan gum, and so forth). However, in producing a suitable fermentation product from whey, technological, market, and economic factors must be considered. Currently, none of the existing whey fermentation processes have wide-scale use in the dairy industry.

Cheese whey can be used for producing calcium magnesium acetate (CMA) for use as a road deicer. From 10 to 14 million tons of road salt are used annually in the United States and Canada. Salt is an extremely effective snow and ice control agent, and is relatively inexpensive. However, a recent study in New York State showed that, although a ton of road salt costs only $40, it causes more than $1400 in damage (*2*). Salt is corrosive to concrete and metals used in the nation's infrastructure. Salt also is harmful to vegetation and poses an environmental threat to ground water quality in some regions. The Federal Highway Administration has long recognized this problem and recently has identified CMA as one of the most promising alternative road deicers (*3*).

CMA is a mixture of calcium acetate and magnesium acetate. It is currently being manufactured by reacting glacial acetic acid with dolomitic lime (Ca/MgO) or limestone ($Ca/MgCO_3$). CMA has a deicing ability comparable to salt, but is noncorrosive and harmless to vehicles, highway concrete, bridges, and vegetation. It is biodegradable and has no identified environmental concerns. However, at the present cost of $650/t vs $40/t for salt, CMA is too expensive for widespread use, even though some studies have shown that all of its material cost can be offset by the savings in infrastructure replacement costs. Consequently, CMA is currently used only in limited areas where corrosion control is required or in environmentally sensitive areas to protect vegetation from salt poisoning (*4*). Using CMA as a deicer, however, will be widely accepted if its production costs can be reduced to $300/t ($0.15/lb) or less (*5*).

CMA's high cost is mainly attributed to the acetic acid used to produce CMA. Currently, the acetic acid for industrial purposes is obtained principally from natural gas. In 1989, the US production of acetic acid was 3.8 billion lb (*6*). Many attempts have been made to ferment biomass as an alternate route to producing acetic acid or CMA. In general, three fer-

Table 1
Comparisons of Various Fermentation Routes to Produce Acetate from Glucose

| | Aerobic vinegar fermentation | *C. thermoaceticum* | Anaerobic digestion |
|---|---|---|---|
| Acetate yield | <60% | >80% | 30%~80% |
| Acetate concentration | ~10% | <3% | <3% |
| Fermentation time | 1~2 d | 1~7 d | 6~15 d |
| Energy requirements | High in aeration and agitation | High in product recovery | High in product recovery |

mentation routes have been studied for their potential use to produce acetic acid or acetate from biomass. The characteristics of these fermentation methods are summarized in Table 1.

The traditional acetic acid (vinegar) fermentation involves two steps: (1) fermentation of sugar to ethanol by yeasts, and (2) oxidation of ethanol to acetic acid by species of *Acetobacter*. This aerobic process requires high energy input and suffers from low yields, only about 50% (wt/wt). Consequently, this process is not economical and cannot compete with the natural-gas-based synthetic process (*7,8*).

More recently, much attention has been placed on anaerobic homoacetogens for acetic acid or CMA production (*9*). Among all the homoacetogens known to date, only *C. thermoaceticum* has been previously considered to have industrial applications (*10*) and, thus, was the only one having been extensively studied for anaerobic acetic acid fermentation (*11–17*). However, the results from this bacterium were generally not satisfactory owing to the low acid concentration (<2%) attained in the fermentation broth and the high cost of the feedstock (glucose) used. In order to attain a viable, economical production, acetate concentration must exceed 4% and a low-cost feedstock should be used (*14*).

Anaerobic digestion of biomass as a means of producing CMA in a mixture of organic acid salts was also studied (*18–21*). In this process, growth of methanogens was suppressed to allow acetic accumulation. Sewage sludge (*18,19*), woody biomass (*20,21*), and in principle, any low-grade biomass, such as cheese whey, can be used in this process. However, the reaction rate is extremely low, and acetate yield is only 30~80%, depending on the fermentation condition. The acetate concentration obtained from this process was also very low, only 0.8%, although theoretically 3% is possible (*18*). Other organic acids present in the product stream included propionic and butyric acids. The major problem of using this process is that the reactor performance is not stable because many undefined mixed cultures are involved.

In this work, a novel, anaerobic coculture was developed to produce CMA from cheese whey. Homolactic and homoacetic bacteria were used to convert whey lactose to lactate and then to acetate in a continuous, im-

mobilized cell bioreactor. The acetic acid produced from this fermentation was recovered and concentrated by using a novel extraction process. The advantages of this process include high yield (>90%) and low recovery costs. An integrated fermentation-separation (extractive fermentation) process for further improving reactor productivity is also discussed.

## MATERIALS AND METHODS

### Cultures and Medium

*Streptococcus lactis* and *Clostridium formicoaceticum* ATCC 27076 were used in this study. These cultures were grown in media containing lactose or lactate as the carbon source. The composition and preparation of the medium have been described elsewhere (22).

### Fermentation

Batch fermentations were studied using 2-L fermentors under strict anaerobic conditions. Each batch fermentation was inoculated with 50 mL active culture. Anaerobic conditions were maintained by keeping a positive $N_2$-$CO_2$ gas pressure (5 psig or 1.34 atm) inside the fermentor. Unless otherwise noted, all fermentations were at 35°C and at a constant pH of 7.6, controlled with NaOH, $Ca(OH)_2$, or $Mg(OH)_2$ solution.

Continuous fermentations were studied using 0.5-L column reactors with external recirculation. A spiral-wound fibrous matrix was packed in the column for cell immobilization. The gap between two spiral-wound layers was about the thickness of each fibrous layer. Thus, the reactor was only ~50% filled with the fibrous matrix. Cell immobilization in this fibrous matrix was achieved by cell adsorption to fiber surfaces and entrapment in the matrix. Each reactor was inoculated with 50 mL active culture and then allowed to grow for 2 d before a slow feed was started. The reactors were then given 2–4 wk to allow cells to saturate in the reactor packing. Thereafter, steady-state data at various dilution rates were collected. In general, operation at each dilution rate was continued for at least two-reactor-volume feed or 2 d to allow the reactor to reach steady state. The reactors were maintained at 35°C and pH 7.6.

### Extraction

Acetic acid extraction was carried out with 50% Alamine 336 (Henkel Corp., Minneapolis, MN), which is a $C_8$—$C_{10}$ straight-chain tertiary amine, in 2-octanol. Detailed procedures have been given elsewhere (23).

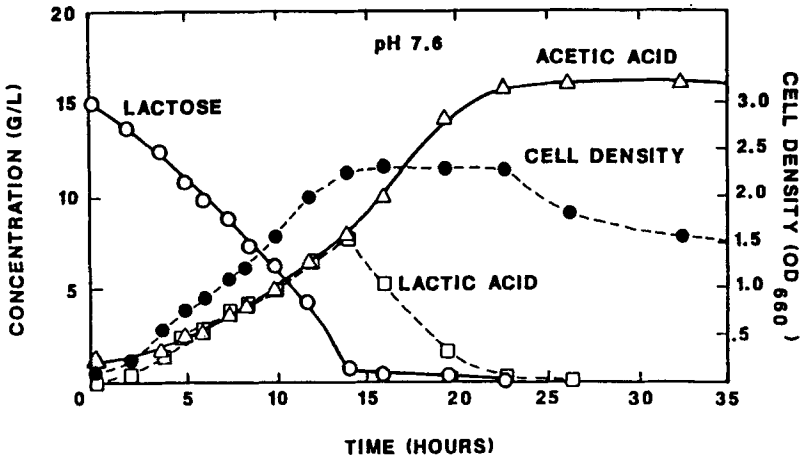


Fig. 1. A cocultured fermentation for acetate production from lactose.

## Assay Methods

Organic compoounds, mainly lactose, lactate, and acetate, present in the fermentation broth were analyzed using high-performance liquid chromatograph (HPLC). Acetic acid in the extraction experiment was measured using gas chromatography. Cell concentration was determined from the optical density at 660 nm ($OD_{660}$) measured with a spectrophotometer. All these assay methods have been described elsewhere (22,23).

# RESULTS AND DISCUSSION

## Acetate Production from Lactose

### *Batch Fermentation*

As shown in Fig. 1, the coculture of *S. lactis* and *C. formicoaceticum* converts lactose to lactate and then to acetate, with >90% (wt/wt) acetate yield from lactose. The pH in this batch fermentation was maintained at ~7.6 by continuous addition of NaOH. It is noted here that the acetate production from lactose also can be carried out in two separate, sequential fermentations. Also, the homoacetogen has a much slower growth rate than the homolactic bacterium. Thus, the rate-limiting step in this fermentation is the conversion of lactate to acetate by *C. formicoaceticum.*

CMA can be produced by mixing dolomitic lime with acetic acid either during or after acetic acid fermentation. Since CMA is the desired final product, it is preferable to use dolomitic lime or CaO/MgO, instead of NaOH, to control the pH during fermentation. The batch fermentation

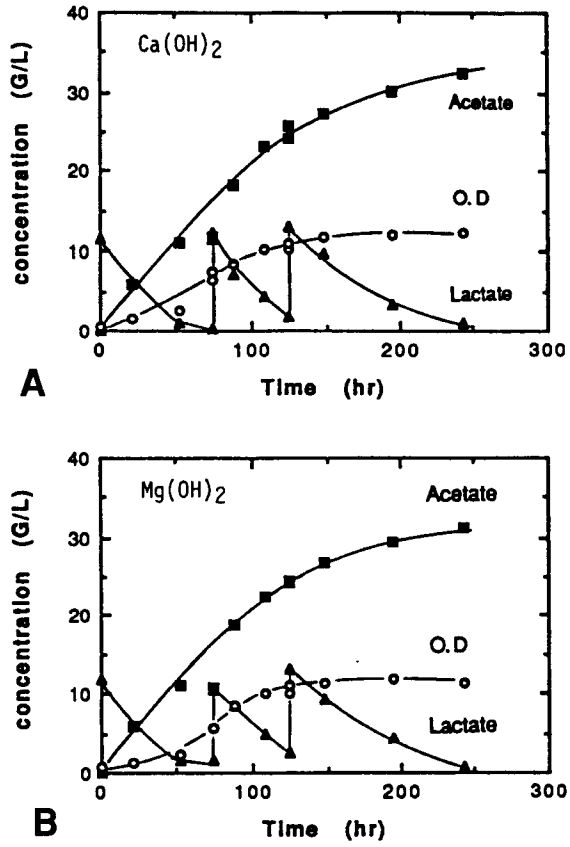


Fig. 2. Batch homoactic fermentation of lactate by *C. formicoaceticum* at pH 7.6—(a) pH control with $Ca(OH)_2$, (b) pH control with $Mg(OH)_2$.

kinetics of *C. formicoaceticum* grown on lactate has been extensively studied in the presence of sodium ion (*24–26*), but not with calcium or magnesium ion. Figures 2(a) and 2(b) show homoacetic fermentations of lactate with $Ca(OH)_2$ and $Mg(OH)_2$ to control the pH, respectively. In these two fermentations, the substrate lactate was replenished twice to allow the fermentations to continue until it was limited by a high acetate or cation concentration. More than 0.5*M* (3%) acetate was obtained at 245 h fermentation time. Although higher acetate concentrations could be attained if more lactate were provided, the fermentation rate would be too slow possibly because of product inhibition. The fermentation rate, however, can be dramatically improved by using immobilized cells.

In general, the fermentation rate was not significantly different for fermentations using $Ca(OH)_2$, $Mg(OH)_2$, or NaOH (data not shown) to control the pH. Therefore, the continuous fermentation was studied using NaOH to control the pH. This was solely owing to the convenience of performing the experiments using NaOH.

*Continuous Fermentation*

In the continuous fermentation study, bacterial cells were immobilized in a spiral-wound, fibrous matrix in the bioreactor. One major advantage of using the immobilized-cell, continuous bioreactor is the high reactor productivity resulting from high cell density. A continuous process using immobilized cell bioreactors has been shown to generate less cell mass and, thus, has lower nutrient requirements while maintaining high product yields. Our reactor was shown to have cell densities >30 g/L. However, cell immobilization in this bioreactor was not irreversible, since constant growth of new cells and sloughing off dead cells occurred within the reactor. The reactor is thus self-renewing and eventually establishes a dynamic steady-state cell population. Therefore, it is possible for the bioreactor to be operated for long periods of time without suffering from problems, such as clogging and high-pressure drop normally observed with conventional packed-bed and membrane bioreactors. The two bioreactors used in this study have been in continuous operation for 6 mo without encountering any contamination or degeneration problems.

Figure 3 shows homolactic fermentation with nearly quantitative conversion of 4.3% lactose to lactate at a retention time of ~28 h. Figure 4 shows homoacetic fermentation with nearly complete conversion of 4.2% lactate to acetate with the reactor retention time around 44 h, which is considerably shorter than those found for batch fermentations with free cells. It is noted that these results were obtained under well-mixed conditions, which may contribute to significant product inhibition. A higher fermentation rate can be attained if the bioreactor is operated under plug-flow conditions. As also shown in Figs. 3 and 4, the product yields in these two fermentations were 98.8 and 96.7%, respectively. Therefore, the overall acetate yield from lactose would be ~95%.

The >90% acetate yield from this homofermentation process is much higher than the 60% or less from the aerobic vinegar process. It also compares favorably with the yields from anaerobic fermentation of glucose using *C. thermoaceticum*, which usually requires much longer fermentation time to attain an acetate concentration of <3%) (Table 2). In addition, this coculture is the only effective way to produce acetate from lactose with high acetate yields. It is noted here that this coculture also can be used to ferment glucose to acetate. The homolactic bacterium *S. lactis* ferments glucose as effectively as it ferments lactose (*27*).

However, the final acetate concentration of 4% obtained from this cocultured fermentation is still low as compared to ~10% from the vinegar fermentation (*28*). This low acetate concentration may lead to prohibitively high energy requirements for concentrating and recovering the acetic acid using conventional recovery methods (*7*). Solvents with high distribution coefficients can extract acetic acid from a low concentration solution more effectively (*29*). These include trioctylphosphine oxide

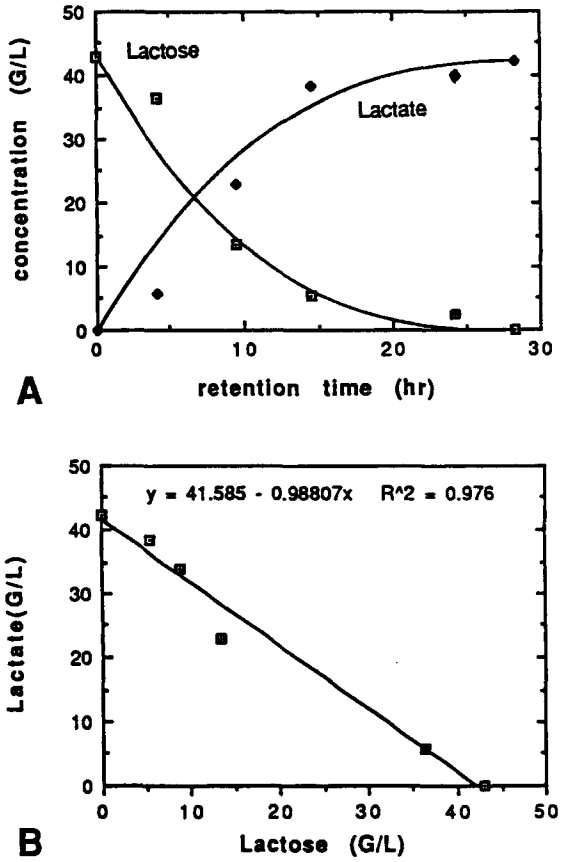


Fig. 3. Continuous homolactic fermentation at various retention times. (a) Lactate production as a function of retention time; (b) lactate yield from lactose as determined from the linear plot of lactate concentration vs lactose concentration.

(TOPO), and tertiary and quaternary amines (*30*). In general, tertiary amines have better physical properties, are less expensive than TOPO and quaternary amines, and were thus chosen for use in this study.

## Acetic Acid Extraction

Acetic acid extraction by using the mixture of 50% Alamine 336 in 2-octanol at various equilibrium pH values was studied. The results are shown in Fig. 5. As shown in this figure, the distribution coefficient, $K_D$, was greatly affected by the pH. In general, the $K_D$ value increased with a decrease in pH and reached a maximum of ~2.5 at pH <4.0. The $K_D$ value was zero at pH >7.5, indicating that Alamine 336 only extracts the undissociated acid and will not work under basic conditions. However, the acetic acid produced in the anaerobic fermentation of pH ~7 is in the form of acetate salt. This problem can be overcome by adapting the microbes to the acidic pH (*14*) or by acidifying the broth before extraction (*31*).

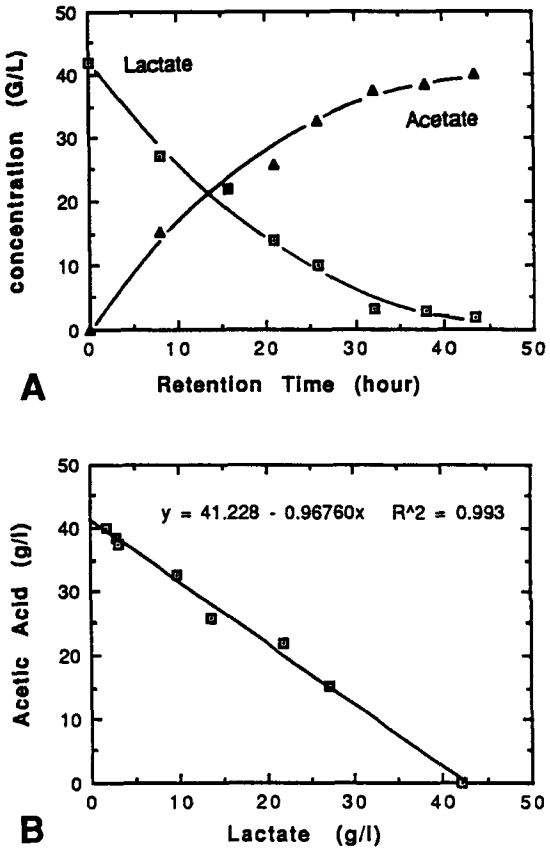


Fig. 4. Continuous homoacetic fermenation at various retention times. (a) Acetate production as a function of retention time; (b) acetate yield from lactate as determined from the linear plot of acetate concentration vs lactate concentration.

Table 2
Acetate Production from Glucose by *Clostridium thermoaceticum*

| Strain | Glucose, g/L | Acetate g/L | Yield,[1] g/g | Time, h | Reference |
|---|---|---|---|---|---|
| ATCC 39073 | 20 | 14.4 | 0.91 | - | *10* |
| S-3 | 21 | 20.0 | 0.98 | 180 | *13* |
| Wood | 20 | 15.0 | 0.83 | - | *13* |
| DSM-521 | 40 | 37.0 | 0.92 | 130 | *15* |
| DSM-521 | 40 | 30 | 0.85 | 28 | *15* |
| ATCC 39289 | 19 | 15.4 | 0.93 | 120 | *17* |
| ATCC 39289*[2] | 35 | 29 | 0.83 | 140 | *17* |

[1]Yield is based on glucose utilization; the actual yields for most cases would be ~80% because not all glucose initially present could be fermented.
[2]Strain adapted to high-acetate environment, formal ATCC no. to be assigned.

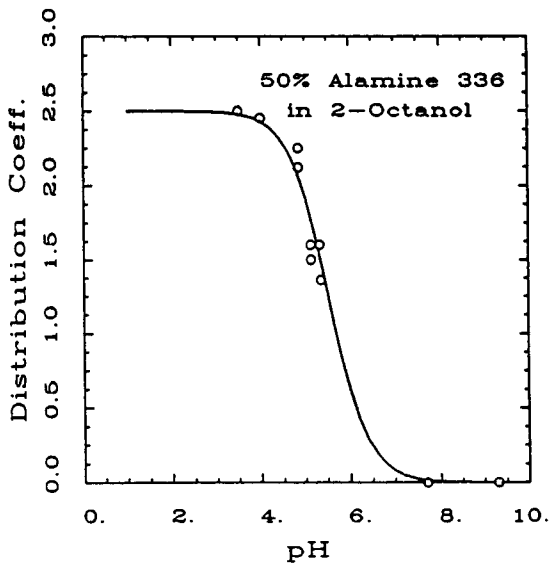


Fig. 5. Effect of pH on distribution coefficient for acetic acid extraction with Alamine 336.

An extractive separation and recovery process normally involves two steps: extraction and solvent regeneration. The amine extractant is only capable of extracting undissociated acid and will not extract acetic acid under basic conditions. This characteristic allows solvent regeneration through back-extraction with an alkaline solution. Therefore, this amine extractant can be easily regenerated by stripping with a small volume of an alkaline solution. In the meantime, the dilute acid solution can be concentrated to an acid-salt solution by this two-step extraction process. The extraction of acetic acid with the tertiary amine is thus highly energy efficient and will provide an economical method for recovering acetate from dilute, aqueous solutions. It is noted, however, that this recovery process is not appropriate for producing pure acetic acid. CMA produced from this extraction process showed comparable deicing ability to CMA produced from pure acetic acid.

The use of 2-octanol as the diluent in the amine extractant increased the $K_D$ value severalfold (*23*). The diluent also improves the physical characteristics of the extractant. However, the optimal composition for the extractant-diluent mixture needs to be further evaluated. $K_D$ value also can be further improved by carefully selecting an appropriate diluent (*31*).

## Extractive Fermentation

The homoacetic fermentation is strongly inhibited by the product, acetate, and possibly the salt added to control the reactor pH. It may be beneficial to integrate the fermentation and the extraction process to remove the acetic acid from the reactor while it is being produced. How-

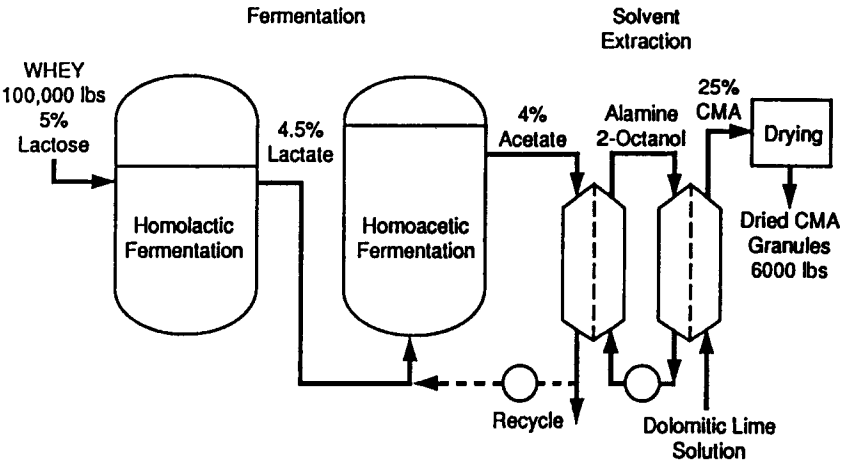


Fig. 6. A fermentation-extraction process for CMA production from whey.

ever, for extractive fermentation, the reactor performance will be highly dependent on the pH. Both the fermentation and extraction are highly pH-dependent. A higher pH favors the fermentation (*26*), whereas a lower pH favors the extraction. Thus, it is essential to understand the effects of pH on the extraction and the fermentation before an extractive fermentation process can be optimally designed. Furthermore, most extractants work efficiently only at acidic pHs, whereas *C. formicoaceticum* requires a pH value >6 to grow (*26*). It is thus important to find an extractant that will work well at a relatively high pH value. It is known that quaternary amines, such as Aliquat 336 (Henkel Corp.), can extract acetate salt (*23*). Also, the homoacetic bacterium may be adapted to grow at lower pH. More work is needed to develop the extractive fermentation process.

## Process for CMA Production from Whey

Based on the laboratory results obtained from this work, a new process for CMA production from whey permeate is shown in Fig. 6. This fermentation process will involve two stages: the first stage for homolactic fermentation and the second stage for homoacetic fermentation. This will allow the two different bacteria to grow at two different optimal conditions. However, depending on the final process design and economics, a one-stage fermentation process with both homolactic and homoacetic bacteria situated in the same bioreactor is also feasible.

After fermentation, the whole broth containing acetate can be directly concentrated (by evaporation) and dried to make CMA. However, if acetate concentration is not high enough or purer CMA is desired, the acetic acid can be recovered and concentrated by using the two-step extraction process shown in Fig. 6. The extraction step will require a packed column extractor to provide multiple contacts necessary to recover 99% of the acetic acid present in the fermentation broth.

The acetic acid present in the extractant can be easily stripped by back-extraction with a small amount of an alkaline (dolime) solution. A simple mixer-settler can be used for back-extraction and reaction to form CMA. A concentrated acetate (CMA) stream is obtained, and the extractant is regenerated, simultaneously. Thus, there is no need to further concentrate the CMA solution before drying. Also, there is no need for expensive distillation to regenerate the extractant.

Also, the second bioreactor, or homoacetic fermentation, can be integrated with the product recovery system to remove the fermentation product, acetic acid, by solvent extraction continuously. Since the produced acetic acid is removed from the reactor, there is no need to add excessive amounts of alkali to maintain the pH. This will minimize the inhibitions caused by the acid product and cations added in controlling the pH. Thus, high fermentation rates can be attained. However, as discussed earlier, further research is needed before such a process can be developed.

CMA, as a road deicer, need not to be as pure as glacial acetic acid used in the chemical industry. Since distillation is no longer needed for further purifying acetic acid or regenerating the extractant, recovery costs for CMA can be reduced significantly. The economics thus become favorable for the production of CMA from whey permeate.

In addition, the waste stream from the CMA production process will be clean, almost free from lactose, acetate, or other organics. Since the solubility of the extractant in water is $<5$ ppm, loss of solvent into the waste stream will be insignificant. The waste stream thus will have very low BOD content and will require minimal treatment, if any, essentially eliminating the waste-disposal problem for the dairy industry.

## Process Economics

An early study conducted by Stanford Research Institute (*9*) estimated that the CMA production costs from corn using *C. thermoaceticum* to ferment glucose to acetate were \$0.266/lb for the 100 t/d plant and \$0.188/lb for the 1000 t/d plant. Depending on the plant size, the raw material costs associated with the feedstock used in the fermentations accounted for $30 \sim 50\%$ of the total production costs. Any costs associated with the raw materials will be transfered to the product cost on a one-to-one basis. It is thus compelling to use waste materials to produce CMA. Also, the CMA production costs were found to be more sensitive to the operating costs than to the capital costs. Increasing the acetate concentration from fermentation is especially important for reducing the operating costs when energy-intensive evaporation and distillation are used in product recovery. Similar conclusions also resulted from a more recent study (*32*).

Another recent study using sewage sludge or woody biomass to produce CMA showed the production costs would be \$0.117/lb if the residue biomass costs \$50/t, or \$0.092/lb if the biomass is free (*19*). Anaerobic

digestion with 6.5-d fermentation time to reach 3% acetate was assumed in the process evaluation. The product yield from fermentation was assumed to be 50%, and solvent extraction was used for acetate recovery and reaction to make calcium acetate. The plant size in this analysis was 500 t/d CMA.

Based on the cost relationships and financial analyses provided from previous studies (*9,19,32*), the CMA production costs could be easily reduced to \$0.09~0.15/lb or less by using zero-cost raw material, such as whey permeate. About 1.2 lb CMA can be produced from each pound of lactose fermented. Therefore, each year, 1.7 billion lb (0.77 million t) of CMA can be produced from the currently unused whey in the nation. This amount represents 5.5~7.7% of the total amount of deicing salt used annually in the North America. Since the market demand for (low-cost) CMA will be big, there should be no problem selling the product. All the currently unused whey, thus, can be converted to a marketable product by using this fermentation process. For a typical, large plant processing 1,500,000 lb/d whey permeate, 90,000 lb CMA, worth \$27,000 at current market price, can be produced. The savings in the disposal costs also will be significant. At least \$2500/d in the disposal costs can be saved by merely avoiding the freight to ship the waste product to other users or the treatment site. It would cost much more if BOD reduction or landfilling were required for disposal, which presently costs \$20/t or higher. The economics for CMA production from cheese whey using the described process is thus very favorable.

## ACKNOWLEDGMENTS

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