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Potassium Acetate by Fermentation with *Clostridium thermoaceticum*

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ABSTRACT

Potassium acetate is currently made by reacting petroleum-based acetic acid with potassium hydroxide. An alternate process, anaerobic fermentation of dextrose with *Clostridium thermoaceticum*, could be used and could possibly be cheaper. Growth characteristics and productivity of the fermentation were optimized to maximize acetate concentration in the broth. The effects of pH, type, and concentrations of nutrients and reducing agents were also evaluated. Corn steep liquor and stillage from an ethanol plant were effective and much cheaper substitutes for yeast extract. Preconcentrating the cells by ultrafiltration improved productivity, resulting in an acetic acid concentration of 53.6 g/L in 50 h at pH 6.5 using corn steep liquor. Sodium sulfide could be substituted for cysteine as the reducing agent with yields greater than 0.9 g acetic acid/g glucose.

Index Entries: *Clostridium thermoaceticum*; potassium acetate; acetic acid; fermentation.

INTRODUCTION

Potassium acetate has been approved by the US Federal Aviation Administration for deicing of airport runways and aircrafts, replacing urea and glycol *(1)*. It is environmentally safe and easily biodegradable *(2)*. It also can be used as a heat transfer fluid in antifreeze formulations and in heat pumps. Currently, potassium acetate is made by reacting petroleum-based acetic acid with potassium hydroxide. An alternate method is to manufacture the acetate from dextrose by fermentation.

Anaerobic fermentation for acetate production by *Clostridium thermoaceticum* has been extensively investigated *(3–15)*. The most attractive fea-

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ture of this fermentation is high acetate yield (theoretically, 1 g/g glucose), which is 50% higher than the two-step fermentation process used for vinegar production. Among the many improvements in the anaerobic fermentation are development of strains with tolerance to acetate concentrations as high as 6–10% *(3–6)*, reduction in nutrient costs *(7,8)* and higher productivity using membrane cell-recycle bioreactors *(9–11)* and immobilized cell bioreactors *(12)*. Much of the prior work focused on sodium acetate or calcium magnesium acetate *(5)*. However, potassium may be more toxic than sodium for strain DSM521 of this micro-organism *(13)*. This paper reports on the fermentation aspects of the process: effects of pH, nitrogen source, reducing agents, and cell concentration on production of potassium acetate with a mutant strain of *C.thermoaceticum*. Downstream processing is being investigated and will be published elsewhere.

MATERIALS AND METHODS

The mutant strain of *C. thermoaceticum* (ATCC 49707 and DSM 6867) was adapted to grow in media containing high concentrations of potassium by transferring it alternatively to the nutrient medium (described below) containing 5% potassium acetate and the medium without any acetate. The medium for culture adaptation and maintenance contained (in g/L) the following components: Glucose = 20; Buffering components ($KHCO_3$ = 9, KH_2PO_4 = 1.4, K_2HPO_4 = 1.1); yeast extract = 5; salts ($MgSO_4.7H_2O$ = 0.25,$(NH_4)_2SO_4$ = 1, $CoSO_4.7.5H_2O$ = 0.03, $Fe(NH_4)_2(SO_4)_2.6H_2O$ = 0.04, $Na_2WO_4.2H_2O$ = 0.0033, $Na_2MoO_4.2H_2O$ = 0.0024, $NiCl_2.6H_2O$ = 0.00024, $ZnSO_4.7H_2O$ = 0.00029, Na_2SeO_3 = 0.000017) and reducing agent (cysteine. $HCl.H_2O$) = 0.25. The medium for fermentation experiments was the same composition, except it contained only 1.8 g/L of $KHCO_3$. The concentrations of glucose, yeast extract (or other nitrogen source), and salts were varied in different experiments. The concentrations given above are referred to as X concentration. When concentrations of yeast extract and salts were two or three times the above concentration, they were referred to as 2X and 3X concentrations, respectively.

The nutrient medium for fermentation was prepared as described previously *(14)*. For batch experiments, the initial glucose concentration was 46–50 g/L. All fermentation experiments were carried out in a 2-L reactor with 1 L working volume. Temperature was controlled at 60°C and the pH was maintained at the desired value by addition of oxygen-free 8N KOH. The fermenter was overlaid with sterile CO_2. Fermentation was initiated by transferring 24-h old inoculum (10% v/v). Fed-batch experiments were conducted as described earlier *(10,17)*: Concentrated solutions of selected nutrients were added at various times during the fermentation.

Glucose and acetic acid were analyzed by HPLC using the Aminex 87H column (Bio-Rad, Hercules, CA). "Acetate" data in this paper refers to

acetic acid with a molecular weight of 60. Thus 1 g of acetic acid = 1.635 g potassium acetate. Cell concentration was monitored by optical density at 600 nm wavelength.

Effect of pH

These experiments were conducted at pH 5.5, 6.0, 6.5, and 7.0. The concentration of yeast extract and salts were at the 2X level.

Effect of Reducing Agents

Various reducing agents were used at concentrations of 0 to 0.25 g/L. Yeast extract (Difco) was used as the complex nitrogen source and pH was kept at 6.5 with KOH. The concentrations of yeast extract and all salts were at the 2X level.

Low-Cost Nutrients

Corn steep liquor was obtained from A. E. Staley Manufacturing (Decatur, IL), as a 50% (w/w) solution. It contained 20% (w/w dry basis) lactic acid. Stillage was obtained from Pekin Energy (Pekin, IL). It is the aqueous suspension remaining after ethanol is steam-stripped away from an ethanol fermentation broth. It contained 7% total solids (mostly dead yeast cells) and 1.47% lactic acid (i.e., 21% lactic acid on a dry basis). Stillage was prefiltered using an ultrafiltration membrane (UFP500, A/G Technology, Needham, MA). The clear permeate from the membrane was used as a nutrient in the fermentation. Solids content was determined by air drying at 80°C for 24 h. Concentrations of nutrients reported are all on dry bases.

Fermentation with High Cell Density

The inoculum was grown in 3 L of medium. It was then concentrated by ultrafiltration to 800 mL. This inoculum with high cell concentration (OD of 15–19) was combined with 200 mL nutrient medium to initiate fermentation.

RESULTS AND DISCUSSION

Effect of pH

In all experiments, the typical fermentation pattern reported earlier *(7,8,11)* was observed: an increase in optical density until the end of the exponential phase, followed by a decrease (Fig. 1). Acetic acid production followed the cell growth curve, but decreased significantly after growth stopped. In some cases, fructose was produced as a by-product, especially when nutrients were insufficient. Table 1 summarizes results of two replicate experiments. With 2X yeast extract and 2X salts, pH 6.5 resulted in the

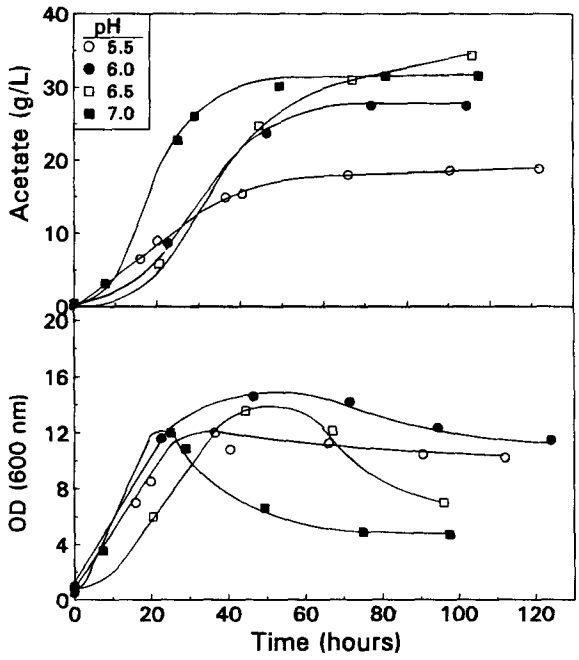

Fig. 1. Effect of pH on growth and acetate production by *C. thermoaceticum* in batch fermentation.

Table 1
Effect of pH on Potassium Acetate Fermentation*

pH	Glucose initial (g/L)	Glucose utilized (%)	Fermn. time (hours)	Acetate concn. (g/L)	Yield (g acetate/ g glucose)	Acetate productivity (g/L.h)	Max. OD
5.5	46 6	42.8	71	17.8	0.91	0 25	10.8
6.0	46.4	80.6	86	31 8	0.89	0.36	14.3
6.5	46.3	90 8	96	33.5	0.84	0 43	12.8
7 0	46.4	90.0	75	31.5	0 81	0.42	12.0

*Average of 2 experiments.

highest acetate concentration (33.5 g/L) and productivity (0.43 g/L.h). Acetate concentration was slightly lower at pH 7.0, but significantly lower at pH 5.5. This is probably because of the inhibitory effect of undissociated acetic acid on the micro-organism *(13)*; acetic acid is more dissociated at higher pH.

However, lower pH resulted in lower acetate concentration (Table 1), but more stable cell concentration (Fig. 1). The latter could be important for fermentation with cell recycle, e.g., a recent study showed that at pH 6.0, *C. thermoaceticum* cells remained active for extended periods and produced

Table 2
Effect of Reducing Agents (Nutrients were Yeast Extract and Salts at 2X Levels)

Reducing agent		Glucose		Acetate	Acetate	Fermn.	Acetate	Max.
Type	Conc. (g/L)	Conc. (g/L)	Utilized (%)	Conc. (g/L)	yield (g/g)	time (hours)	productivity (g/L.h)	OD
Cysteine	0.25	46.3	90.3	34.4	0.86	118	0.35	13.6
	0.20	48 9	96.7	37 9	0.85	119	0.32	13.7
	0.15	46.9	96 3	37.6	0.89	115	0.33	11.9
	0 10	46 2	97.7	40.7	0.96	123	0.33	8.4
	0 05	50.3	96.7	42.4	0 94	114	0.37	7.4
Sodium	0.25	47 2	87.4	36 8	0.91	119	0.30	6 1
thioglycolate	0.20	47.3	85.4	35.5	0.89	115	0 30	5.8
	0 15	48 6	92.9	35.3	0 82	125	0.28	6 7
	0.10	47 5	84.7	34.5	0.88	128	0.26	6 0
	0 05	48 6	91.0	40.5	0.93	140	0.27	4 8
Sodium	0.25	45.8	95 2	39 0	0.95	92	0.43	11.6
sulfide	0 10	47.2	97.5	38 5	0.89	146	0.26	6.1
None	0.00	48.4	80.2	29 5	0.80	92	0.32	7 3

up to 38 g/L acetate in continuous cell recycle fermentation *(11)*. Since high acetate concentration is important in the economics of industrial production, pH 6.5 was selected as the optimum for potassium acetate production. The concentration of acetate obtained with KOH was slightly lower than that obtained with NaOH as a neutralizing agent *(11)*.

Effect of Reducing Agents

In most of our prior research, cysteine·HCl·H_2O (0.25 g/L) was used as a reducing agent and as a source of sulfur that is required by *C. thermoaceticum (15)*. Since cysteine is expensive, studies were conducted with alternate low-cost sulfur-containing reducing agents (Table 2). Interestingly, decreasing cysteine concentration from 0.25 to 0.05 g/L actually improved both the final acetate concentration in the broth (Fig. 2) and the yield (Table 2). At the same time, however, maximum OD in the fermentation broth decreased from 13.6 to 7.4. Thus the increase in acetate yield may be a result of decrease in cell mass yield that allowed more carbon to be channeled into acetate production. Koesnandar et al. *(15)* showed

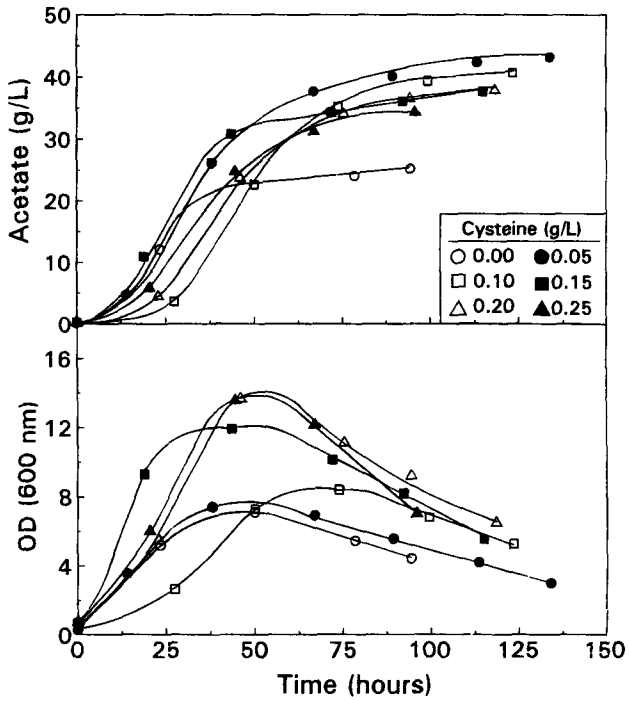

Fig. 2. Effect of cysteine $HCl{\cdot}H_2O$ concentration on growth and acetate production by *C. thermoaceticum* in batch fermentation.

that cysteine increased growth and acetate production when a minimal medium was used in the fermentation. In this study, the fermentation broth contained complex nitrogen sources such as yeast extract that may have made the presence of high cysteine levels unnecessary.

Similar effects were observed when sodium thioglycolate was used as the reducing agent (Table 2). The lowest sodium thioglycolate concentration resulted in the highest acetate yield and the lowest optical density. With sodium thioglycolate, cell growth was highest at 0.15 g/L and lowest at 0.05 g/L. However, with sodium sulfide ($Na_2S{\cdot}9H_2O$), decreasing its level from 0.25 to 0.1 g/L resulted in lower cell growth and productivity, although acetate level was 38–39 g/L. Our results differ from those of Koesnandar et al. *(15)* who used a minimal medium consisting of mineral salts and vitamins, probably because our medium contained a complex nitrogen source. In addition, the mutant strain used in the present study appeared to be much better since it routinely achieved much higher acetate concentrations (35–40 g/L vs 15 g/L in their study).

A certain concentration of sulfur-containing reducing agent is necessary. With no reducing agent, acetate concentration was only 29.5 g/L, the yield was lower and the maximum OD was only 7.3. Since sodium sulfide is the cheapest of the three reducing agents studied, it could be used in commercial fermentation at a level of 0.1 g/L.

Table 3
Effect of Stillage Concentration

Stillage (g solids per liter)	Glucose initial (g/L)	Glucose utilized (%)	Fermn. time (hours)	Acetate Conc (g/L)	Yield (g/g)	Product-ivity (g/Lh)	Max. OD
5	46.5	74.4	106	23.9	0.70	0.22	5.0
10	46 9	63 2	115	23.5	0.82	0.20	5.9
15	46 7	63.0	118	23.4	0 81	0.19	4.9
20	46 2	67.6	141	28.1	0.92	0 19	6.6
30	46 6	81 3	139	33 6	0.92	0 24	8.2

Low-Cost Nutrients

Because of the high cost of yeast extract, alternate low-cost nutrients were evaluated for the production of potassium acetate. Since most of the glucose in the United States is produced from corn, the focus was on by-products from the corn wet milling industry.

Stillage

As shown in Table 3, stillage concentrations of 5–15 g/L (dry solids basis) resulted in. acetate concentrations of only 23–24 g/L. Higher levels, e.g., 30 g/L, resulted in higher acetate concentration of 33.6 g/L (Fig. 3). Traces of fructose were observed in all experiments. However, yields of acetate were good, especially at high stillage levels, probably because of the conversion of lactic acid in the stillage to acetic acid. Lactic acid constituted 21% w/w (dry basis) of the stillage solids. Thus, stillage is not only a good low-cost nutrient, but also provides an additional carbon source for producing acetic acid.

Corn Steep Liquor (CSL)

Corn steep liquor had previously been shown to be a good nutrient source for *C. thermoaceticum (7,8)*, especially in combination with ammonium sulfate. Figure 4 shows fermentation patterns with CSL. With CSL at 5 g/L, increasing ammonium sulfate concentration from 3 to 5 g/L resulted in an increase in acetate concentration from 26.9 to 33 g/L (Table 4). In general, acetate productivities were higher with CSL (0.27–0.32 g/L·h) than with stillage, but not as good as with yeast extract.

The fermentation could be improved by using the fed-batch mode and an excess of nutrients. With CSL at 13.5 g/L and ammonium sulfate at 2.7 g/L, an acetate concentration of 38.8 g/L (Fig. 4B) and acetate yield of 1.02 g/g were obtained. The high yield is probably a result of utilization of lactate in CSL by the organism to produce additional acetate.

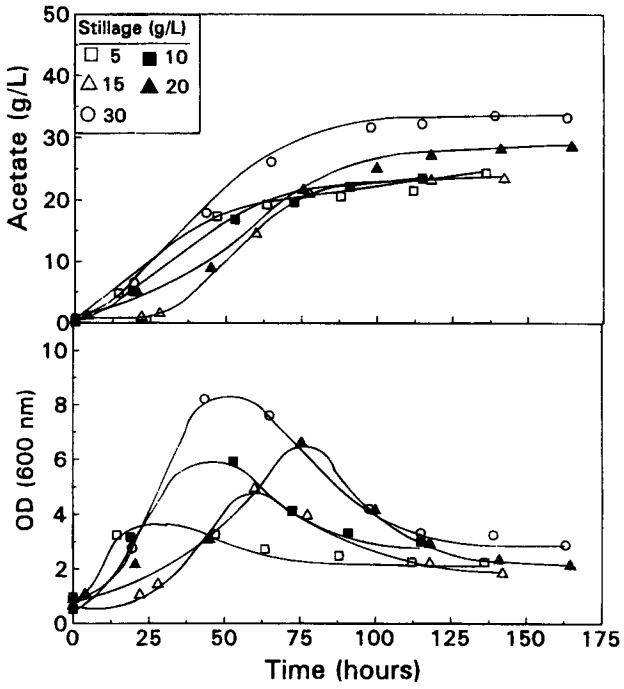

Fig. 3. Effect of filtered stillage concentration on growth and acetate production by *C. thermoaceticum* in batch fermentation.

Table 4
Batch and Fed-Batch Fermentation with Corn Steep Liquor (CSL)*

Experiment	CSL (g/L)	AS[a] (g/L)	Salts[b]	Glucose Conc. (g/L)	Glucose Utilized (%)	Fermn time (hours)	Acetate Conc. (g/L)	Acetate yield (g/g)	Acetate productivity (g/L.h)	Max OD
Batch	5.0	3.0	2X	46.5	86.5	100	26.9	0.70	0.27	8.9
	5 0	4 0	2X	46 6	93 6	96	30.2	0 81	0.32	9 2
	5.0	5 0	2X	47.5	87.7	118	33.0	0 83	0.28	7.9
Fed-Batch	13.5	2.7	2.7X	46.3	95.1	129	38 8	1 02	0.30	10 5

*Average of 2 experiments.
[a]AS = Ammonium sulfate.
[b]Does not include ammonium sulfate.

Effect of Cell Density

Productivity can be improved by increasing cell density in the fermenter *(10,11)*. This is shown in Table 5 with preconcentrated cells in media containing either yeast extract (YE) or CSL as the nutrient source. Productivities were higher (0.65–0.71 g/L.h) compared to fermentations

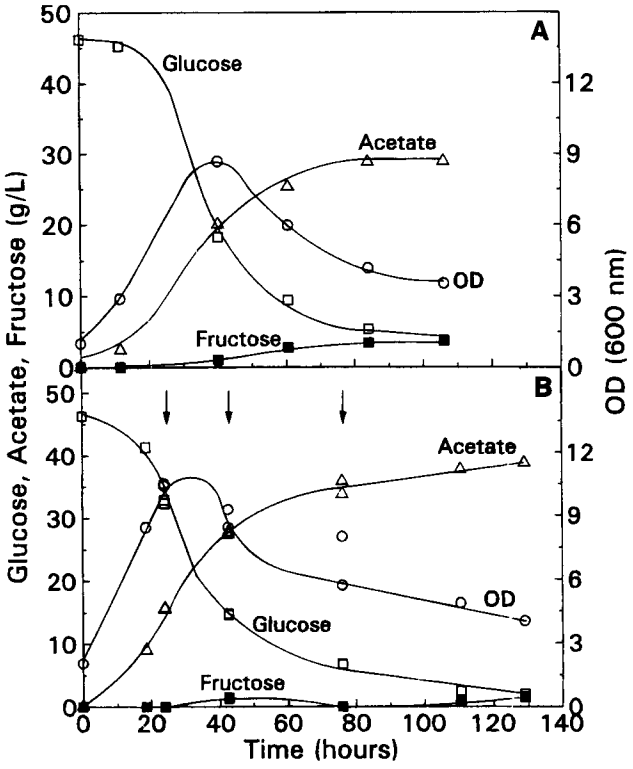

Fig. 4. **(A)** Batch fermentation with CSL = 5 g/L, salts = 2X, ammonium sulfate = 3 g/L. **(B)** Fed-batch fermentation with CSL = 13.5 g/L, salts = 2.7X, ammonium sulfate = 2.7 g/L. Arrows show the times of nutrient addition.

Table 5
Effect of Cell Density on Fermentation Parameters

Expt	Nutrient Type[a]	Nutrient Conc (g/L)	AS[a] (g/L)	Salts[b] (g/L)	Glucose Conc. (g/L)	Glucose Utilized (%)	Acetate Conc (g/L)	Acetate yield (g/g)	Acetate productivity (g/L.h)	Max. OD
High cell density	Yeast extract	7.5	4.0	2.5X	71.6	86.1	50 3	0.74	0.67	22.1
		10 0	6.0	3X	67.8	87.2	48.9	0.82	0.67	19.1
	CSL	5 0	5.0	2X	61 5	84.2	46.4	0 82	0.50	27 7
		10.0	4 0	2X	83 7	81.3	53 6	0.81	0.72	19.4
Low cell density	CSL	5.0	5.0	2X	47 5	87 7	33 0	0 83	0 28	7.9
		10.0	4.0	2X	47.0	77.7	29.2	0.83	0.22	9.5

[a]AS = Ammonium sulfate; CSL = Corn steep liquor.
[b]Does not include ammonium sulfate.

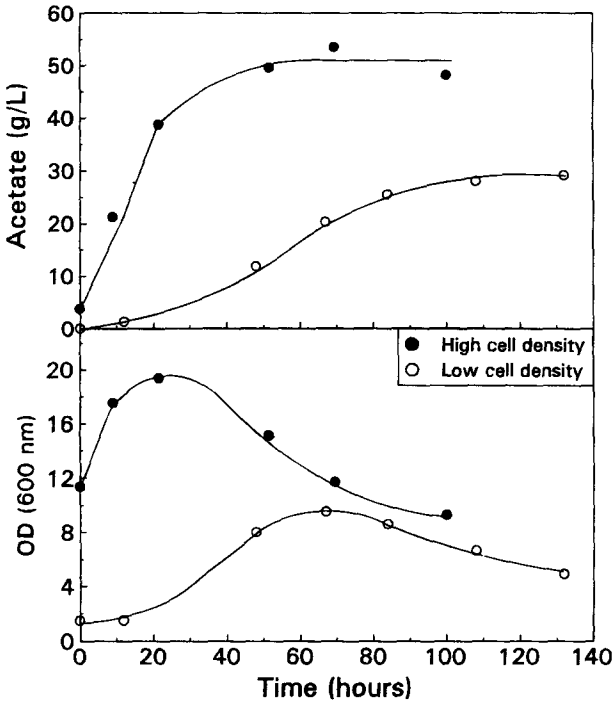

Fig. 5. Effect of cell density on growth and acetate production by *C. thermoaceticum* in batch fermentation. Medium contained CSL = 10 g/L, salts = 2X, ammonium sulfate = 4 g/L.

with normal cell levels (0.22–0.28 g/L.h). The maximum OD values and final acetate concentrations were also much higher. Figure 5 compares these fermentation patterns with the same nutrients but different cell densities. Another benefit of high cell density culture was elimination of the lag period at the start of the fermentation.

In summary, this study has identified the optimum levels of some of the important fermentation parameters for production of potassium acetate from dextrose by *C. thermoaceticum*. The materials cost can be substantially reduced by using sodium sulfide instead of cysteine as the reducing agent, and CSL or stillage as low-cost complex nutrient sources. Fermentation costs can be reduced by preconcentrating the cells, resulting in higher productivity, and higher acetate concentration.

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