Enzyme and Microbial Technology 120 (2019) 8–15

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/enzmictec

A novel low pH fermentation process for the production of acetate and propylene glycol from carbohydrate wastes

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ARTICLE INFO

Keywords:
Immobilized fermentation
Lactose degradation
Lactobacillus plantarum
Lactobacillus buchneri
Deicers

ABSTRACT

A novel low pH fermentation process was studied for the conversion of lactose using *Lactobacillus plantarum* and *Lactobacillus buchneri* under anoxic conditions in single co-culture, and two-stage sequential fermentations. This is aimed at producing acetate and propylene glycol (PG) as environmentally benign substitutes for currently used road and aircraft deicing chemicals. The results indicate that in the case of two-stage fermentation with immobilized *L. buchneri* in the second stage, lactose degradation rate increased markedly producing acetate and PG concentrations of 12.1 and 10.7 g L^{-1} at pH 3.8. In the case of coculture fermentation, the acetate and PG concentrations were 8.2 and 6.8 g L^{-1}, respectively. Fermentation of lactose and whey powder was conducted at pH 4.25 using a high cell density culture of *L. buchneri*. The acetate and PG yields were similar for both substrates at ~0.3 g/g and ~0.33 g/g respectively. With a starting lactose concentration of 60 g/L, acetate and PG concentrations of 18 g/L and 21 g/L respectively were obtained. The low pH conversion of wastes to value-added products under anoxic conditions provides substantial operating benefits over neutral pH fermentations that require strict anaerobic conditions for effective operation. Moreover, the low product pH at around 4.0 will provide substantial savings in downstream processing costs due to the much higher extraction efficiency of weak- and moderate- base resins for acetic acid compared to acetate ion.

1. Introduction

Roads and highways in cold weather countries typically receive applications of deicing chemicals during winter storm events to provide better traction for vehicles, and thereby prevent accidents [1]. Rock salt or sodium chloride is commonly used for moderately low temperatures (−7 °C and above), while calcium and magnesium chloride salts are used for very low temperature conditions (−15 °C and below). While sodium chloride is an effective and low-cost deicer, it has significant deleterious effects on the environment, and it causes significant long-term damages to the highway infrastructure [2,3]. Infrastructure damages stem from the accelerated corrosion of steel bridges and reinforcing steel in concrete pavements, and spalling of concrete surfaces. Salt is not biodegradable, and hence, there is a long-term potential for its accumulation in receiving waters and groundwater. This will affect drinking water quality due to increased sodium levels, and also affect ecosystem stability of ponds and lakes due to increased salinity.

Calcium magnesium acetate (CMA) is a non-corrosive and biodegradable deicer with effective deicing capabilities. It has low toxicity to aquatic organisms, and hence has minimal potential impacts on the environment [4]. The cost of road salt ranges from $69 to $85 per ton [5], whereas CMA cost has ranged from $1,492/Ton [6] to $1,896/Ton [7]. However, even at this relatively high cost, CMA is being used by several state agencies, especially on new bridges, and in environmentally sensitive areas including the Yellowstone National Park and Aspen, CO, and Snowmass Village, CO.

Calcium magnesium acetate is currently produced by reacting synthetic acetic acid with dolomite and concentrating and drying the product. Dolomite is abundantly available in nature and is inexpensive. Acetic acid is mainly produced by the carbonylation of methanol, a high pressure and high temperature process that results in high product costs and significant greenhouse gas emissions [8]. Hence the potential production of acetic acid using biobased processes using agricultural raw materials and biomass wastes has been of particular interest. The use of biomass wastes with zero to negative costs, will reduce the upstream raw material costs, and also reduce environmental impacts by avoiding waste disposal issues.

Acetic acid has been produced since ancient times using the two-step vinegar process (yeast fermentation to produce alcohol and aerobic oxidation of ethanol using *Acetobacter*). Theoretical yield of ethanol from glucose is 0.51 g/g. Actual yields are 80%–90% of the theoretical yield. Actual yield of acetic acid from ethanol is ~1.2 g/g ethanol.

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https://doi.org/10.1016/j.enzmictec.2018.09.006
Received 31 August 2018; Received in revised form 18 September 2018; Accepted 20 September 2018
Available online 21 September 2018
0141-0229/

Thus, the net yield of acetic acid is ~0.5 g/g glucose. Fermentation time for the two stage fermentation ranges from 192 h to 288 h [9,10]. However, aerobic oxidation requires the supply of oxygen, and since the pH of fermentation is around 6–7, extraction of acetic acid from the broth is inefficient and costly.

Most anaerobic bacteria can produce acetic acid [11]. Marynowski et al. [12] evaluated a production process for acetic acid and CMA based on C. *thermoaceticum* fermentation, but no industrial application was reported due to the low product concentration obtained. A mutant strain of C. *thermoaceticum* (ATCC 49707 and DSM 6867) was studied by Shah et al. [13] for the production of potassium acetate deicer from glucose. Glucose utilization was only 43% at pH 5.5 and reached a maximum of 91% at pH 6.5. The fermenter was overlaid with sterile CO_2, and cysteine was added to maintain anaerobic conditions. The reactor was maintained at 60 °C. The maximum acid productivity obtained in this case was only 0.43 g/L/h. Parekh and Cheryan [14] used a cell-recycle membrane reactor with C. *thermoaceticum* for the conversion of glucose to acetate. Acid productivity ranged from 1 g/L/h to 4 g/L/h for a glucose feed of 30 g/L. However, the acid yield ranged from only 0.55 to 0.63, and the acetate concentration was only 16 g/L for the higher productivity rate. Since *C. thermoaceticum* is an obligate anaerobe, it is hard to maintain such strict anaerobic conditions in a production process (100% CO_2/N_2 environment in every step from inoculum preparation to fermentation). For CMA production at pH > 6.0, dolomite is not reactive enough to dissolve readily. This pH is the lower limit at which *C. thermoaceticum* can function [12]. Also, the high operating temperature of ~60 °C requires energy supply and results in loss of sugars by reaction between glucose and phosphate [15].

Yang et al. [16] used *C. formicoaceticum* and *Lactococcus lactis* in a two-step anaerobic process to produce acetate from whey, in two separate, sequential fermentations. The acetate yield was ~95% at a product concentration of ~45 g/L. When mixed fermentation was conducted at pH 7.6, a product concentration of 20 g/L was obtained in 20 h, and production slowed thereafter due to inhibition, and a final acetic acid concentration of 30 g/L and residual lactate concentration of 20 g/L was reached after 80 h. These strains are also obligate anaerobes, and require maintenance of strict anaerobic conditions. Moreover, the acid productivity is low, and the bacteria are inhibited by low pH conditions. Nevertheless, detailed economic analysis by these authors indicate that CMA can be produced at a cost of $215/Ton using this process [17]. This estimation is in close estimation by Pal and Nayak [18] and Chukwu and Cheryan [19], the production cost of acetate from fermentation of biomass wastes for CMA is about $0.3–0.5 per kg. Currently, CMA made from synthetic acetic acid is being sold at a price of $0.75–$0.8 per kg.

Talabardon et al. [20] conducted free cell and immobilized cell fermentations of lactose to produce acetic acid using *C. thermolacticum* and *M. thermoautotrophica* at a pH of 6.4, and temperature of 58 °C. With free-cell fermentation the acetate yield was only 0.46 g/g lactose, and the highest final acetate concentration obtained was 15 g/L. Ethanol was co-produced with a yield of 0.3 g/g. The acid productivity was only 0.54 g/L/h. As evident from the above studies, one of the basic problems with these anaerobic fermentations is the inability of the bacteria to acclimate to low pH and/or moderate to low acetate concentrations in the production process.

Schwartz and Keller [21,22] investigated methods to isolate *C. thermoaceticum* strains that can produce acetic acid at a pH ~ 4.5 based on their conclusion that a fermentation pH < 4.5 would be necessary for the economical production of biobased acetic acid. They isolated a strain of *C. thermoaceticum* that can produce acetic acid at pH 4.5 through acclimation by decreasing pH from 6.0 to 4.5 in a stepwise manner. The highest concentration of free acetic acid attained was 1.4 g/L, and the total acetic acid concentration was 2.3 g/L. The mass doubling time was 36 h at pH 4.5 compared to 5 h at pH 6. Schwartz and Keller [22] compared the growth of four strains of *C. thermoaceticum* at pH 6 and 7 under different initial acetic acid concentrations. At acetic acid concentrations greater than 10 g/L, two strains failed to grow, and the doubling time increased from 5 to 7 h to 18 h for the remaining strains. They concluded that free acetic acid is more inhibitory to *C. thermoaceticum* than low pH conditions or the acetate ion. As reported by several researchers, the cost of extraction of acetic acid from the fermentation broth is quite high at the normal anaerobic fermentation pH conditions of ~7 compared to the cost of extraction of the unionized acid at pH ~ 4 [23–25]. Hence, there are no commercial anaerobic acetic acid production facilities at present [26].

Fu [27] has shown that *Lactobacillus plantarum* is able to ferment lactose over a pH range 4–7, though the conversion rate and efficiency was reported to be maximum at pH > 5. Elferink et al. [28] have shown that *Lactobacillus buchneri* can degrade lactate to acetate under anoxic conditions effectively at acidic pH values in the absence of an electronic acceptor. Carvalho et al. [29] have reported that *L. buchneri* (LB) is not able to ferment sugars such as lactose, galactose, mannose etc., and hence LB could not be directly used in lactose fermentation. Tolerance to high product acid levels along with their ability to ferment under anoxic or microaerophilic conditions make *L. plantarum* (LP) and LB suitable for consideration for commercial applications [27,28,30,31].

Two-stage fermentation process, coculture fermentation, or single-stage direct fermentation are suitable approaches that can be used in the conversion of waste lactose to acetic acid. Several researchers have investigated the coculture of LP and LB and other *Lactobacillus* sp. in silage fermentation [32–34]. These authors have concluded that inoculation of LP plus LB not only improves the aerobic stability of silage, but also was able to produce ≥13 g acetic acid kg^{-1} silage while lowering the levels of lactic acid in comparison to the un-inoculated controls. Reich and Kung [33] reported about 29 g kg^{-1} DM and 12 g kg^{-1} DM of acetic acid and 1,2-propane diol, respectively, while lowering ethanol levels from corn silages at pH 3.58. Elferink et al. [28], proposed a novel pathway for anaerobic degradation of lactic acid by LB into equimolar amounts of 1, 2-propanediol and acetic acid, and trace amounts of ethanol. Since, 1,2-propane diol, commonly known as propylene glycol (PG) is used as the principal agents in aircraft deicing and anti-icing fluids [35], it is expected that a coculture or two-stage process using LP and LB would be attractive for large-scale production of these chemical deicing agents.

The previous studies that have been conducted to investigate CMA production were typically under strict anaerobic conditions and at pH ~ 7 [16,36]. There have been no studies reported in the literature on coculture fermentation of LP and LB strains under controlled conditions to determine fermentation kinetics and process efficacy. Much of the reported studies have focused on aerobic stability of silages, and these studies lasted for 2160–6000 h [30,37]. Such long reaction times would not be feasible for bioreactors designed to operate on a large scale for the production of organic acids. This study aims to address these shortcomings through the study of fermentation kinetics of LP and LB under low pH (pH ~ 4) conditions for the conversion of synthetic whey waste, and whey permeate powder, both containing lactose as the major carbon source for acetate and PG production.

2. Materials and procedure

2.1. Microorganisms and growth conditions

L. plantarum (ATCC 21028) and *L. buchneri* (ATCC 4005) obtained from American Type Culture Collection (Manassas, VA, USA) were used in the current work. The culture was maintained in the De Mann-Rogosa-Sharpe (MRS) media supplemented with 20% glycerol (v/v) at −25 °C. The culture was routinely tested for viability and cultured in MRS medium incubated at 36 °C under static anaerobic conditions.