Succinic Acid Production from Cheese Whey using *Actinobacillus succinogenes* 130 Z

Caixia Wan · Yebo Li · Abolghasem Shahbazi · Shuangning Xiu

Received: 15 May 2007 / Accepted: 27 August 2007 / Published online: 22 September 2007 © Humana Press Inc. 2007

**Abstract** *Actinobacillus succinogenes* 130 Z was used to produce succinic acid from cheese whey in this study. At the presence of external CO₂ supply, the effects of initial cheese whey concentration, pH, and inoculum size on the succinic acid production were studied. The by-product formation during the fermentation process was also analyzed. The highest succinic acid yield of 0.57 was obtained at initial cheese whey concentration of 50 g/L, while the highest succinic acid productivity of 0.58 g h⁻¹ L⁻¹ was obtained at initial cheese whey concentration of 100 g/L. Increase in pH and inoculum size caused higher succinic acid yield and productivity. At the preferred fermentation condition of pH 6.8, inoculum size of 5% and initial cheese whey concentration of 50 g/L, succinic acid yield of 0.57, and productivity of 0.44 g h⁻¹ L⁻¹ were obtained. Acetic acid and formic acid were the main by-products throughout the fermentation run of 48 h. It is feasible to produce succinic acid using lactose from cheese whey as carbon resource by *A. succinogenes* 130 Z.

**Keywords** Succinic acid · Cheese whey · Lactose · Fermentation · *Actinobacillus succinogenes*

**Introduction**

Succinic acid, known as amber acid or butanedioic acid, is a four-carbon dicarboxylic acid produced as an intermediate of the tricarboxylic acid cycle (TCA) [1, 2]. Succinic acid and its derivative have wide industrial applications such as the feedstock of food and pharmaceutical products, as the intermediate of chemical synthesis of surfactants, detergents, green solvents, and biodegradable plastics, and also as ingredients of animal feeds to stimulate animal and...
plant growth [2–5]. Currently, most of commercial succinic acid is produced through petrochemical process, which brings environmental pollution and the concerns of sustainable development [2, 3]. The production of succinic acid by microbial fermentation is a simple and environmentally friendly process [4, 6, 7]. However, to date, bio-based succinic acid is not yet competitive with petrochemical-based acid, mainly owing to high production cost [8]. There is a need to develop cost-effective conversion technology to produce succinic acid from renewable resource such as food processing waste [9–11].

Many anaerobic and facultative anaerobic microbes produce succinic acid as the fermentation end product [5]. *Actinobacillus succinogenes* 130Z, originally isolated from bovine ruminal contents, is a facultatively anaerobic, capnophilic and Gram-negative bacteria, which has been considered as the most potential succinic acid producer to produce a significant amount of succinic acid from glucose under anoxic condition [12]. This strain 130Z also showed distinctive ability to convert a broad range of carbon sources such as arabinose, cellobiose, fructose, xylose, and reduced sugar to succinate as the major end product and acetate, formate, lactate, and ethanol as the minor end products [13]. This strain has an advantage over other previously reported succinic acid producers because it can tolerate the presence of high concentration of succinic acid or its salt [14].

It was reported that *A. succinogenes* 130Z and its variant strain FZ6 produced 66.4 and 105.8 g/L of succinic acid with the yield of 0.67 and 0.8 from glucose, respectively, which indicated *A. succinogenes* had huge potential to be developed as a commercial succinic acid producer [13, 14]. Continuous and repeat-batch biofilm fermentation of succinic acid by strain 130Z demonstrated a significant increase in succinic acid productivity (7 g h⁻¹ L⁻¹) and yield (86.7%) [15]. Environmental and physiological studies showed that CO₂ level and pH were the most critical factors affecting both cell growth and succinic acid formation. Increase in CO₂ supply and electron donor resulted in increase of succinic acid production and less formation of by-products such as ethanol and formate. This is most likely due to the increased PEP carboxylation to oxaloacetate rather than PEP conversion to pyruvate, where the pathway was regulated by the level of CO₂ and electron donors [16].

Whey is produced as a by-product during cheese making and as a potentially environmental pollutant due to its high biological oxygen demand (BOD) [17]. Whey consists mainly of 6 to 7% solids, of which 70 to 80% is lactose and 10 to 15% soluble proteins, lactate, and other mineral salts [11]. It can be directly used as feed additive and also has a continuing interest to be alternatively utilized as the low-cost substrate to produce value-added biochemicals such as lactic acid [17, 18]. Previous studies showed that *Actinobacillus succiniciproducens* and *Mannheimia succiniproducens* can ferment whey directly into succinic acid [11, 19]. However, the studies concerning the fermentation of succinic acid from cheese whey using *A. succinogenes* have not been reported.

The objectives of this study were to develop fermentative protocol for succinic acid production from cheese whey by *A. succinogenes* and study the effect of environmental and nutritional factors such as external CO₂ supply, pH, inoculum size, and initial whey concentration on succinic acid production.

**Materials and Methods**

**Organism and Growth Conditions**

*A. succinogenes* 130Z (ATCC 55618) was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in 250 mL sealed anaerobic bottles