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Enhanced succinic acid production from corncob hydrolysate by microbial electrolysis cells

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HIGHLIGHTS

- An MEC bioreactor was used to produce succinic acid from corncob hydrolysate.
- The MECs could supply more reducing power for *A. succinogenes*.
- Combining $Ca(OH)_2$ and NaOH pretreatment of hydrolysate was benefit to the MECs.
- The improved succinic acid production by using NaOH as a pH regulator.

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ABSTRACT

In this study, *Actinobacillus succinogenes* NJ113 microbial electrolysis cells (MECs) were used to enhance the reducing power responsible for succinic acid production from corncob hydrolysate. During corncob hydrolysate fermentation, electric MECs resulted in a 1.31-fold increase in succinic acid production and a 1.33-fold increase in the reducing power compared with those in non-electric MECs. When the hydrolysate was detoxified by combining $Ca(OH)_2$, NaOH, and activated carbon, succinic acid production increased from 3.47 to 6.95 g/l. Using a constant potential of −1.8 V further increased succinic acid production to 7.18 g/l. A total of 18.09 g/l of succinic acid and a yield of 0.60 g/g total sugar were obtained after a 60-h fermentation when NaOH was used as a pH regulator. The improved succinic acid yield from corncob hydrolysate fermentation using *A. succinogenes* NJ113 in electric MECs demonstrates the great potential of using biomass as a feedstock to cost-effectively produce succinate.

1. Introduction

Succinic acid is one of the most important platform chemicals, and it can be used as a precursor for many chemicals of industrial importance, such as adipic acid, 1, 4-butanediol, and tetrahydrofuran (Song and Lee, 2006; Zeikus et al., 1999). For sustainable development in this era of petroleum shortages, the production of succinic acid via the microbial conversion of renewable feedstock has attracted great interest (McKinlay et al., 2007a,b). Among succinic acid-producing strains, *Actinobacillus succinogenes* has great potential because it can efficiently convert different carbon sources to succinic acid (Guettler et al., 1999). Du developed a wheat biorefining strategy for succinic acid production using *A. succinogenes* (Du et al., 2008). Liu used *A. succinogenes* to produce succinic acid using cane molasses as a low-cost carbon source (Liu et al., 2008a). Chen used a diluted acid hydrolysate of corn fiber as a carbon source for *A. succinogenes* to produce succinic acid (Chen et al., 2010), and Kim used a wood hydrolysate-based medium to culture *Mannheimia succiniciproducens* for the production of succinic acid (Kim et al., 2004).

However, the pentose reducing power is not sufficient in such hydrolysates. For example, the reducing power output is 1.67 mol of NADH per mol xylose or arabinose, which is 16.5% less than that of glucose, when these sugars are catabolized anaerobically (Iverson et al., 2013; Lin et al., 2005). Compared with glucose, more NADH (Nicotinamide Adenine Dinucleotide Hydrogen) is necessary for xylose to achieve a high yield of reductive products. To improve the balance of [H] reducing power, MECs have been used (Li et al., 2009; Rozendal et al., 2008). MECs can transfer anode electrons to a mediator and, subsequently, to microorganisms, which compensates for the shortage of reducing power (Min et al., 2009; Park et al., 1999). Butyric acid production increased by 34% when *Clostridium tyrobutyricum* BAS7 was used in electrochemical bioreactor system with neutral red as the

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electron carrier (Park et al., 1999). At the same time, acetic acid was converted to ethanol using a mixed microbial community as a cathode of a bioelectrochemical system (Steinbusch et al., 2010). In addition, alcohol was produced by a mixed culture when volatile fatty acids were reduced using hydrogen as an electron donor (Steinbusch et al., 2008). Additionally, the conversion of fumarate to succinic acid has been achieved using *A. succinogenes* with electrically reduced neutral red as an electron donor in electrochemical bioreactor system (Park et al., 1999). It was also shown that the level of NADH increased and the rate of butanol production increased by 53% when *Clostridium acetobutylicum* was used in a three-electrode potentiostatic system (Peguin and Soucaille, 1996).

In this study, the effect of MECs on succinic acid production using a corncob hydrolysate as a carbon source was evaluated, and three different corncob hydrolysate pretreatment methods were investigated. Furthermore, different voltages were optimized in the MECs to ensure efficient electron transport. Finally, fermentation of a corncob hydrolysate for succinic acid production was developed using different pH regulators strategies in MECs. The present study is the first to demonstrate that using MECs to improve the reducing power in corncob hydrolysate can efficiently enhance succinic acid production by *A. succinogenes* NJ113.

2. Methods

2.1. Chemicals

Yeast extract (YE) was from Oxoid Ltd. (Cambridge, UK). Neutral red and dithiothreitol (DTT) were from Aladdin (N108710 and D104860, respectively; Shanghai, China). 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT), phenazine methosulfate (PMS), and yeast ADHII were from Aladdin (Shanghai, China). Other chemicals were of reagent grade and were from either Sinochem (Shanghai, China) or Fluka chemical (Buchs, Switzerland). Carbon dioxide (CO_2) was from the Nanjing special gases factory (Nanjing, China).

2.2. Microorganism and media

A. Succinogenes NJ113 (China General Microbiological Culture Collection Centre, CGMCC NO. 1716) was used in all experiments.

The medium for the inoculation cultures was composed of 10 g glucose L^{-1}, 5 g YE L^{-1}, 9.6 g $NaH_2PO_4{\cdot}2H_2O$ L^{-1}, 15.5 g $K_2HPO_4{\cdot}3H_2O$ L^{-1}, 10 g $NaHCO_3$ L^{-1}, 1 g NaCl L^{-1}, and 2.5 g corn steep liquor L^{-1}. Glucose was aseptically added to the medium after autoclaving.

2.3. Growth condition and cell preparation

The inoculation medium was inoculated with 1 mL of a glycerol stock culture that was stored at −70 °C, and it was incubated at 37 °C, with shaking at 11.5*g*, under anaerobic conditions. Resting cell suspensions were prepared by harvesting stationary phase cultures at 4 °C by centrifugation at 8000*g* for 5 min. The cells were washed twice with 50 mM phosphate buffer (pH 7.0) containing 1 mM DTT. The washed cells were resuspended in catholyte, and then removed to the cathode of the MECs.

2.4. Microbial electrolysis cells

MECs were specially designed for cultivating strictly anaerobic bacteria. They were made from Pyrex glass by the Chemistry Department, Michigan State University (East Lansing, MI, USA). The MECs were separated into anode and cathode compartments by a cation-selective membrane septum (Nafion; diameter, 35 mm; 5.6 Ω cm^{-2} when measured in 0.25 M NaOH (Lancaster, N.Y.). No chemicals or metabolites can be transferred across the Nafion membrane except protons or cations. The anode and cathode were made from fine carbon felt (5 mm thick; 10.6 cm^2 g^{-1} available surface area). The electrodes were connected to a power supply (model 1825; Cole-Parmer, Vernon Hills, IL, USA) with a platinum wire (diameter, 1 mm; 1.0 Ω cm^{-2}; Sigma–Aldrich, St. Louis, MO, USA). The platinum wire was connected to the carbon-felt electrodes with graphite epoxy (1.0 Ω cm^{-2}). The total volume in each MEC compartment was 300 ml, and the liquid volume was 280 ml. The weight of both electrodes was adjusted to 1.1 g (surface area, 12 cm^2). For *A. succinogenes* experiments, the anode and cathode compartment headspaces were separated and filled with CO_2, and the gas phases were equilibrated by continuous circulation at a 25 ml/h flow rate. The cells were grown in the cathode compartment. The catholyte initially contained (per liter): 5.0 g YE, 10.0 g $NaHCO_3$, 8.5 g $NaH_2PO_4{\cdot}2H_2O$, 31.5 g $NaHPO_4{\cdot}12H_2O$, and 0.0288 g Neutral red; 35.8 g/l $NaHPO_4{\cdot}12H_2O$, 15.6 g $NaH_2PO_4{\cdot}2H_2O$, 5.8 g NaCl, and 0.02% DTT were used as the analyte. Prior to sterilization, the initial pH of the analyte was adjusted to 7.2 using 10 M NaOH. DTT and Neutral red were filtered through a 0.22-μm syringe filter prior to use.

Constant potential was defined as the potential of an electrode relative to a reference electrode (Ag/AgCl) (Park et al., 1999).

Standard hydrogen electrode (SHE) was defined as the universal reference electrode, which has a standard electrode potential (that is, at pH 0) of 0 V (Park et al., 1999).

2.5. Preparation of corncob hydrolysate

Corncobs with a moisture content of 5.6% (w/v) were obtained from Shandong Zhengde Foods Ltd., China. The corncobs were ground with a commercial plant grinder and passed through 20–40-mesh screens before mixing with 2% (v/v) sulfuric acid at a ratio of 1:5 (w/v). The suspension was hydrolyzed in an autoclave at 121 °C for 20 min.

Method one and two were that the raw hydrolysate was adjusted to pH 7.2 with alkaline $Ca(OH)_2$ and NaOH, respectively. Method three was that $Ca(OH)_2$ was added to the raw hydrolysate for neutralizing H_2SO_4, and concentration of $Ca(OH)_2$ was added at equal mole concentration of H_2SO_4. Then NaOH was used to adjust pH to 7.2. And any solid material was removed through filter paper. Activated carbon was used to remove inhibitors. Activated carbon 2% (w/v) was added to the corncob hydrolysate, and the mixture was heated at 50 °C for 30 min, and then passed through filter paper to remove the activated carbon.

2.6. Analytical methods

The dry cell weight (DCW) was computed from a curve relating the optical density at 660 nm (OD_{660}) to the dry weight. An OD_{660} of 1.0 represented 520 mg of dry weight per liter (Xi et al., 2012).

The concentrations of sugars and organic acids were measured by high-performance liquid chromatography (Agilent 1290; Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent model variable wavelength detector (VWD) for organic acids and refractive index detector for sugars, and an HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA, USA). The column was operated at 55 °C. The mobile phase was 0.008 M sulfuric acid at a flow rate of 0.6 mL/min. The injection volume was 20 μl.

The intracellular concentrations of NADH and NAD^+ were measured using a cycling method according to the literature (Leonardo et al., 1996) and as following. Ten-milliliter samples were removed from aerobic and anaerobic cultures, and the dinucleotides were extracted by a modification of the protocol described by Heber