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Incorporation of CO_2 during the production of succinic acid from sustainable oil palm frond juice

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ABSTRACT

The utilization of the juice from the cheapest carbon source oil palm frond (22 $/ton) in a 3.5 L bioreactor was proven to be competent in replacing pure sugar in succinic acid production. The current study provides an in-depth discussion on the utilization of CO_2 in the production of succinic acid. In the serum-bottle fermentation, the yield of succinic acid increased by 51% from 0.47 to 0.71 g/g while the final titer of succinic acid enhanced by 4 fold were attributed to the anaplerotic reaction of *Actinobacillus succinogenes* and the alkalinity effect of the dissolved CO_2. In the bioreactor, the highest mass transfer in the fermentation medium was achieved at 0.5 vvm CO_2 at which 3 M KOH was pumped in at a rate of 2.0 mL/min to neutralize the carbonic acid formation, which afforded the highest succinic acid concentration of 30.7 g/L. A first report on actual stoichiometry equation relating substrate, CO_2 consumption and all products formation was derived from the results of bioreactor fermentation to lay as a guideline for future reference of actual succinic acid production reaction. $C_6H_{12}O_6 + 0.145\ NH_3 + 0.642\ CO_2 \rightarrow 1.083\ C_4H_6O_4 + 0.509\ CH_2O_2 + 0.420\ C_2H_4O_2 + 0.117\ C_2H_5OH + 0.726\ CH_{1.8}N_{0.2}O_{0.5} + 0.614\ H_2O$. Actual stoichiometry equation revealed that for every kg of succinic acid produced, 0.22 kg of CO_2 will be absorbed. The desirable effect of CO_2 on the production of succinic acid may translate into a mutually-beneficial relationship between the succinic acid and the CO_2-generating biofuel industries.

1. Introduction

The rising concerns for climate deterioration and sustainability have drawn global efforts to shift the production of chemicals through the petrochemical approach to biotechnological pathway [1]. In this regard, biofuels and biochemicals have drawn much attention owing to the increasing awareness of the adverse consequences of the reliance on fossil fuels [2]. Succinic acid, for instance, has conventionally been produced through the petrochemical approach [3]. However, it has been listed by the U.S. Department of Energy as one of the 12 chemical building blocks with the potential for commercial production through biological methods [4]. The commercialization of bio-succinic acid has been realized since 2012, with at least 12 production plants operating worldwide by 2014 [5]. The production of bio-succinic acid is superior to the conventional petrochemical production in terms of not only sustainability but also environmental friendliness. Independent life-cycle analysis conducted by BioAmber in one of their production plants for bio-succinic acid reported a net zero carbon footprint alongside a reduction of 60% in total energy consumption compared to the petrochemical counterpart approach [6]. Carbon footprint of biobased succinic acid is 0.85 kg CO_2 eq/kg, including the footprint reducing effect of CO2 uptake by the corn-based succinic acid of 1.49 CO_2 eq/kg, and less than half as high as the carbon footprint of fossil-based succinic acid as much as 1.8 kg CO_2^- eq/kg [7].

The biofuel industry is thriving with vast potentials. In 2013, there were over 14,500 biomethane plants in Europe with an installed capacity of 7857 MW [8]. Besides Europe, this industry was forecasted to be fast-growing in the U.S., Brazil and Asia in the near future [9]. Biohydrogen is likewise projected to grow increasingly popular, especially with the recent announcement on the launch of Hyundai Tucson, Toyota Mirai and Honda Clarity fuel-cell vehicles which consume hydrogen fuels [10]. Japan is planning on the commercialization of biomass-based hydrogen production [11] to support the advent of hydrogen fuel-cell vehicles. Another fast developing CO_2-producing biofuel industry was bioethanol which has been commercialized in many countries, especially in the U.S. and Brazil [12]. The proliferation

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of these CO_2-producing biofuel industries could have mutualism relationship with CO_2-consuming succinic acid production industry. The CO_2-rich gas generated from these industries could be used as the source of CO_2 to succinic acid production plant.

The utilization of CO_2 in the production of chemicals and fuels is gaining global interest [13]. The presence of CO_2 is critical in the production of succinic acid through anaplerotic reaction of the microbe. High availability of CO_2 promoted the flux towards the C4 succinic acid-producing pathway [14] since the fixation of CO_2 into the three-carbon PEP was needed to form the four-carbon oxaloacetate (OAA). Apart from that, McKinlay and Vieille [15] have reported the detection of the decarboxylation of OAA and malate into pyruvate. However, the presence of CO_2 could suppress such decarboxylation of OAA and malate [15]. The decarboxylation of C4 to C3 intermediate was sufficiently high to exert a pronounced effect on the yield of succinic acid. Thus, the presence of CO_2 critically affected the yield. These decreasing fluxes from OAA/malate to pyruvate coupled with the increasing carboxylation of pyruvate to malate resulted in higher net flux into C4-pathway and thus augmenting the final yield of succinic acid [14].

Carbon source is the largest component in fermentation which has a direct impact on the overall cost of production. The oil palm plantation in Indonesia and Malaysia produces a massive amount of residue which could replace costly pure sugars in several biochemical industries. The greatest residue from oil palm plantation was oil palm frond (OPF) (70%), amounting to roughly 83 MMT annually in Malaysia [16]. OPF was viewed as the cheapest potential source of carbon source for bio-succinic acid industry [17]. These residues have significant economic potentials which were overlooked. The worldwide production of OPF was estimated at a weight of 250 MMT per annum judging from the ratio of global oil palm plantation (13 million ha) [18] to Malaysia oil palm plantation of 4.49 million ha [19]. Biomass Technology Centre (BTC) was set up by Malaysia Palm Oil Board (MPOB) in 2001 targeting at researching ways to produce high value added products from oil palm biomass and promote the image of oil palm industry as an environmental friendly industry through zero waste approach [20]. Bio-production of succinic acid using oil palm biomass in this regard, is in line with the mission of BTC. Moreover, Malaysia Prime Minister has made a pledge at the 15th Conference of Parties of the United Nations Framework Convention on Climate Change in Copenhagen to reduce the GHG emissions intensity per Gross Domestic Product (GDP) by 40% by 2020 [20], which is another driving incentive for using oil palm biomass as bio-succinic acid production feedstock.

The objective of this study was to investigate the effect of carbon dioxide (CO_2) on two scales: one in serum-bottle fermentation and another in bioreactor-based fermentation. Different carbonate loadings were used in serum bottles to investigate their impact on the succinic acid yield by *Actinobacillus succinogenes*. Direct sparging of CO_2 was used in the bioreactor-based fermentation to investigate the relationship between the diffusivity and maximum productivity of succinic acid production. An actual stoichiometry equation relating all products related in succinic acid production has been developed. The desirable effect of CO_2 on the production of bio-succinic acid may translate into a mutually-beneficial collaboration between the succinic acid-producing industry and the CO_2-producing biofuel industries, including bioethanol, biomethane and biohydrogen industries. The ability of OPF juice to replace pure glucose in bioreactor fermentation was investigated in this paper. The utilization of economic juice from OPF as a sustainable source of carbon could elevate the economic feasibility of bio-succinic acid industry.

2. Materials and methods

2.1. Microorganism

A. succinogenes DSM 22257 was acquired from the German Collection of Microorganisms and Cell Cultures (DSMZ). The bacterium was revived and inoculated in brain heart infusion medium containing brain heart infusion broth (1.75%, w/w) and peptone (1.00%, w/w) at 37 °C and 120 rpm over a period of 8 h before it was added into the fermentation medium at the ratio of 1:10 [21].

2.2. Media composition

All the chemicals used for the media preparation were of reagent grade, and were obtained from Ever Gainful Enterprise (Selangor, Malaysia) unless otherwise stated. The formulation of the fermentation media supplemented was (per liter of water) $CaCl_2$, 0.2 g; KH_2PO_4, 3.0 g; NaCl, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; Na_2CO_3, 20.0 g; yeast extract, 15.0 g; and antifoam B emulsion (Sigma-Aldrich, St. Louis, USA), 1.0 g [22]. D-glucose solution at 40 g/L was sterilized separately, after which it was mixed with the fermentation medium in sterile condition.

2.3. Fermentation in serum vials

The anaerobic fermentation of D-glucose was performed in 100-mL serum bottles aseptically. The serum-bottle fermentation was conducted in Labfors Incubator Shaker from Bioinfors Company, Switzerland at 37 °C and 200 rpm. The serum-bottle fermentation was conducted at 0, 100, 200, 300, 400 to 500 mM of sodium hydrogen carbonate, $NaHCO_3$ loading to study the impact of carbonate loading on the yield of succinic acid. Each batch of serum-bottle fermentation was run in duplicates.

2.4. Fermentation in bioreactor

In the bioreactor-based fermentation, the sparging of CO_2 was varied from 0.1, 0.3, 0.5, 0.7 and 0.9 vvm to elucidate its effect on the diffusivity of CO_2 and productivity in a 3.5-L Labfors Bioreactor from Bioinfors Company, Switzerland. The effect of different sparging rates on gas-liquid mass transfer was discerned from the rate at which 3 M KOH was pumped in to maintain the pH at 6.8. The anaerobic bioreactor fermentation was conducted at 37 °C and 200 rpm.

2.5. Fermentation of oil palm frond juice

Oil palm fronds (OPF) were freshly harvested from the Universiti Kebangsaan Malaysia oil palm plantation estate in Bangi, 43,600 Selangor, Malaysia. Only the petiole part was taken for juice extraction while the leaflet was left in the estate for nutrient preservation and erosion prevention. The OPF petiole was pressed using a 6.5 HP, SCM model, Elephant brand sugarcane machine was used to extract the juice. The chemicals added were the same as in 2.2 except that the carbon source glucose was replaced by the readily fermentable sugars in OPF juice. Optimized CO_2 sparging rate from this study were used in the fermentation of OPF juice while other conditions remained the same.

2.6. Analytical methods

The sugars were analysed through Agilent 1200 HPLC system (California, USA). This HPLC system was equipped with a Refractive Index Detector (RID) and a Rezex RPM (Phenomenex, USA) column measuring 300 mm × 7.8 mm which was set at 60 °C. Deionized water was eluted at a flow rate of 0.6 mL/min isocratically. The analysis of metabolites including formic acid, acetic acid, succinic acid and ethanol were conducted in an Agilent 1100 HPLC system (California, USA) which was equipped with an ultraviolet detector (UVD) at 210 nm and a Rezex ROA column (Phenomenex, USA). Isocratic elution of 0.005 N H_2SO_4 was carried out at a rate of 0.5 mL/min and at 60 °C [23]. The bacterial growth was quantitatively analyzed by the difference in the dry weights of the samples after the carbonate salts were dissolved using 0.2 M HCl.