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Review

Biological production of adipic acid from renewable substrates: Current and future methods

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ABSTRACT

Adipic acid has been widely used for producing thermoplastic polyurethane resins, nylon 6-6, adhesives, synthetic lubricants and plasticizers, and the global market for adipic acid is estimated at approximate $6.3 billion per year. Currently, 2.85 million tons of adipic acid are produced from petrochemical sources, mostly by oxidation of KA oil (cyclohexanol and cyclohexanone) catalyzed by nitric acid. The chemical synthesis of adipic acid is of serious consequences such as greenhouse gases and heavy pollution to the environment, etc. The researchers are struggling to establish more reliable, renewable and affordable adipic acid synthesis methods, and among which, biological synthesis of adipic acid is attracting the most attention. There are two biological routes for adipate synthesis: (1) biological accumulations of precursors of adipic acid such as D-glucaric acid and *cis,cis*-muconic acid, which can be further catalyzed to adipate; (2) synthesis of adipate directly from carbon source. With the emergence of metabolic engineering and synthetic biology, biological synthesis of adipic acid from renewable substrates is now feasible. Here, we review the new approaches and technologies to produce adipic acid biologically, with the direct synthesis of adipic acid from the renewable substrates of special interest.

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1. Introduction

Adipic acid (hexanedioic acid) with a molecular mass of 146.14 g mol^{-1} and pK_a values of 4.43 and 5.41, is one of the most important aliphatic, straight-chain dicarboxylic acid for commercial purpose [1]. Adipic acid is primarily used for manufacturing nylon 6-6, and the global market of which is estimated at approximate $6.3 billion per year. The forecast of adipate production growth is 4.1% annually, leading to a global production of 3.3 million tons in 2016 [2].

In 2004, in a report published by the Department of Energy of U.S., 12 bio-based chemicals, with a higher market price than biofuels were identified of the greatest market value in the United States [3]. That report has spurred tremendous effort making these bio-based chemicals economically feasible [4]. Currently, the only economically feasible option to produce adipic acid is through petrochemical routes. This option is extremely detrimental to the environment with the massive production of carbon dioxide/nitrous and nitrogen oxide. Most of adipic acid is produced by oxidation of KA oil (cyclohexanol and cyclohexanone) catalyzed by nitric acid [5] (Fig. 1A). Cyclohexanol and cyclohexanone are both produced from benzene and this process generates nitrous/nitrogen oxides, carbon monoxide and other hazardous compounds [6–12]. The adipic acid production in the US generated approximate 1.9 TgCO_2 Eq in 2009 [13]. The researchers are struggling to find a more reliable, renewable and affordable adipic acid synthesis methods, among which, biological synthesis of adipic acid is attracting much attention [9,14,15].

Biologically producing adipic acid was estimated to be 20–30% of total production in the long run [16]. Despite the higher market price and a lot of efforts to synthesize adipic acid biologically, no bio-based adipic acid production methods are economically feasible yet [2,14]. The greatest hurdle of producing adipic acid biologically is that there were no reliable native-occurring adipate synthesis pathways found in microorganisms yet. However, with the fast growth of metabolic engineering and synthetic biology, there are more and more methods available to screen metabolic pathways, integration of orthogonal pathways into host microorganisms and balance metabolic flux [17–23]. A lot of efforts have been done to search for orthogonal enzymes in nature, which might contribute to adipate synthesis. In this article, we primarily review the two biological routes for adipate synthesis: (1) biological accumulations of precursors of adipate such as D-glucaric acid and *cis,cis*-muconic acid, which could be catalyzed to adipate [9,15,24]; (2) biological synthesis of adipate directly from carbon sources [25–28]. Moreover, how to use consolidated bioprocessing (CBP) to directly convert lignocellulosic biomass to adipic acid is also heavily discussed here (Table 1).

2. Production of adipic acid from petrochemicals

Despite the heavy pollutions caused by adipic acid production in the industry, adipic acid is mainly produced from benzene, a toxic chemical. Benzene was first reduced to cyclohexane [10,29–31], which was oxidized under high temperatures (125–165 °C) and high pressure (8–15 atm) to produce KA oil (a mixture of cyclohexanone and cyclohexanol) [32]. Then, the KA oil reacted with nitric acid and air to produce adipic acid, catalyzed by vanadium or copper. The process scheme is illustrated in Fig. 1A. The disadvantages of the current industrial process include low overall product yield (4–11% yield); corrosion of reaction vessels by nitric acid; emission of greenhouse gas N_2O; and high energy consumption [31]. It was estimated that 0.3 kg-N_2O/kg-adipic acid was produced in this process [33].

There is a “green” route for synthesizing adipic acid from cyclohexane by oxidation hydrogen peroxide in a halide free condition [11,34]. This process is described as a “green” route because it did not produce the greenhouse gases. This process is illustrated in Fig. 1C. Recently, a N_2O-free process for adipate synthesis has been reported [31] (Fig. 1B). In a simple experiment, ozone gas was bubbled through cyclohexane with concurrent UV irradiation at room temperature. There was no metal catalyst or solvent involved in this method. After 2–8 h, adipic acid was produced.

Several downfalls exist with both the traditional production route as well as the “green” route. First, all processes still rely on petrochemicals [11,32,34]. Second, while hydrogen peroxide, ozone and UV light are generally safe (fewer hazards associated with production), the prepration process of the starting materials in all three processes are more harmful than the production of adipic acid from the starting material, hence the green routes lead to limited improvements on the environment.

3. Conversion of bio-based precursors to adipate

Until recently, biosynthesis of adipic acid as the final product from carbon substrates has not been heavily studied [2]. However, using the biologically derived precursors such as *cis,cis*-muconic acid or D-glucaric acid, followed by chemo-catalytic conversion process successfully produced adipic acid [9]. This process is currently advantageous because *cis,cis*-muconic acid and D-glucaric acid could be produced based on fermentation platforms [5,9,19,35–41].

3.1. *Metabolic engineering of* Escherichia coli *for* cis,cis-*muconic acid production from* D-*glucose*

Biological production of *cis,cis*-muconic acid has been heavily studied and there are two major production routes: (1) direct synthesis of *cis,cis*-muconic acid from glucose; (2) metabolizing aromatics to *cis, cis*-muconic acid. The second route is of a great importance because the lignin is the major source of aromatics in the nature.

Direct production of *cis,cis*-muconic acid from glucose was first demonstrated in *E. coli* by Draths and Frost in 1994 [42]. Because there were no existing pathways converting D-glucose to *cis,cis*-muconic acid, a synthetic pathway was introduced into *E. coli* (Fig. 2A). The first intermediate of this synthetic pathway was 3-dehydroshikimic acid (DHS) from an advanced shikimate pathway in *E. coli* [43,44]. To increase intracellular accumulation of DHS, a shikimate dehydrogenase-deficient *E. coli* strain hosting the plasmids expressing transketolase, 3-deoxy-*d*-arabinoheptulosonate 7-phosphate (DAHP) synthase, 3-dehydroquinate (DHQ) synthase, DHS dehydratase (AroZ), protocatechuate decarboxylase (AroY) and catechol 1,2-dioxygenase (*cat*A) was engineered [9,37,38,42,45] (Fig. 2A). Finally, 10 g/L *cis, cis*-muconate was produced from 10 g/L D-glucose, and it was then catalyzed to adipic acid in the unpurified cultures with 90% yield [42]. At that time, the biotechnological tools were limited, and the exogenous genes were not codon optimized for *E. coli*. Thus, the yield of *cis,cis*-muconate was far from the theoretical yield. After 8 years, the same group published another milestone paper, in which they significantly increased *cis,cis*-muconate's yield and titer [9]. The *aro*Z gene was integrated into *E. coli*'s chromosome, while the *aro*Y gene and *cat*A gene were harbored in the plasmid. After fermentation optimizations, the final titer of *cis,cis*-muconic acid was 36.8 g/L with 0.22 mol/mol yield from glucose [9]. However, the major drawback of *E. coli* was its low tolerance to the organic acids. As a comparison, eukaryotes, especially *Saccharomyces cerevisiae* was a much better platform for organic acid production. The *cis,cis*-