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Key technologies for the industrial production of fumaric acid by fermentation

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ABSTRACT

The growing concern about the safety of food and dairy additives and the increasing costs of petroleum-based chemicals have rekindled the interest in the fermentation processes for fumaric acid production. The key problems of the industrial production of microbial fumaric acid are reviewed in this paper. Various strategies, including strain improvement, morphology control, substrate choice, fermentation process and separation process, are summarized and compared, and their economical possibilities for industrial processes are discussed. The market prospects and technological strategies for value-added fumaric acid derivatives are also addressed. The future prospects of microbial fumaric acid production are proposed at the end of this article.

Contents

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1. Introduction

Due to the depletion of conventional oil and the deterioration of the global environment, biotechnology that utilizes renewable raw materials and moderate process conditions is considered to be the most promising option to address vital resource and environmental issues. With biotechnology, many chemicals that were produced solely by chemical processes from petroleum-based materials in the past now have the potential to be produced from biomass (Ji et al., 2011). Fumaric acid and its derivatives are examples of such chemicals.

Fumaric acid is a four-carbon dicarboxylic acid that is 1.5 times more acidic than citric acid. Therefore, it is commonly used as a food acidulant and beverage ingredient (Yang et al., 2011). Additionally, fumaric acid is widely used in the feed industry as an antibacterial agent and a physiologically active substance. Fumaric acid has a double bond and two carboxylic groups and therefore can be polymerized to produce synthetic resins and biodegradable polymers (Roa Engel et al., 2008). As an important platform chemical, fumaric acid is a valuable intermediate in the preparation of edible products, such as L-malic acid and L-aspartic acid (Goldberg et al., 2006). With the increasing market share of L-aspartic acid and L-malic acid in sweeteners, beverages and other health food areas, the worldwide demand for fumaric acid and its derivatives grows each year.

The ability of *Rhizopus nigricans* to produce fumaric acid was discovered by Ehrlich as early as 1911. Later, by surveying 41 strains from eight genera of *Mucorales*, Foster and Waksman (1938) identified *Rhizopus*, *Mucor*, *Cunninghamella* and *Circinella* species that are able to produce fumaric acid. Among these strains, *Rhizopus* species were shown to be the best producers of fumaric acid. Industrial-scale production of this compound with *Rhizopus* has been considered by several companies since the 1940s. In 1943, Pfizer disclosed a process for producing fumaric acid under submerged aerobic fermentation conditions with *R. nigrican* (Kane and Amann, 1943). Later, *R. arrhizus* was successfully used by Pfizer to commercially produce fumaric acid, with an annual production of 4000 tons (Roa Engel et al., 2008). National Distillers and Chemical Corporation also had great interest in microbial fumaric acid production. In 1958, this company disclosed a process that used nickel ions to promote fumaric acid production by *Rhizopus* (Lubowitz and La Roe, 1958). They later described improvements in fumaric acid yields after limiting the concentration of nitrogen sources during fermentation (La Roe, 1959). However, this process was discontinued in the 1970s and replaced by chemical synthesis due to the more economical method of chemical synthesis from petrochemical feedstock. Nevertheless, the continuous rise of petroleum prices and the increasing concern about food and dairy safety, as well as the low-carbon economy, have resulted in a resurgence of interest in fumaric acid production by fermentation (Goldberg et al., 2006). In 1986, Du Pont disclosed a process that used fatty acid esters to improve the production of fumaric acid by *Rhizopus* (Goldberg and Stieglitz, 1986). Du Pont later reported an additional strategy to improve fumaric acid production by changing the dissolved oxygen concentration during fermentation (Ling and Ng, 1989). In recent years, Chinese companies such as Changmao Biochemistry and Jiangsu Jiecheng Bioengineering have also focused on microbial fumaric acid production and have invested substantial financial and human resources into fumaric acid industrialization. Although there have been numerous investigations into the development of fermentation processes for fumaric acid production, this process unfortunately remains less efficiency than other commercial organic acid production (Table 1), and the industrialization of microbial fumaric acid production is still in the testing stages.

Fungal production of organic acids, including fumaric acid, has been previously reviewed (Goldberg et al., 2006; Magnuson and Lasure, 2004; Roa Engel, et al., 2008). These reviews either focus on the microorganism its metabolic pathways or give a general description about fermentation process control. The intent of this review is to describe our current understanding of the issues that may be involved in the industrialization of microbial fumaric acid production and to provide a comprehensive overview on the state-of-the-art developments and technological achievements or obstacles in microbial fumaric acid production, such as strains, substrates, fermentation techniques, downstream processes and derivatives.

Table 1
The performances of different organic acid fermentation processes.

Product	Production (g/L)	Productivity (g/L/h)	Source of data
Citric acid	140–160	> 2.0	Industrial process
Lactic acid	170–180	> 3	Industrial process
Succinic acid	100–120	~1.8	Pilot scale
Fumaric acid	30–50	0.4–1.0	Article reported

2. Biochemistry and physiology

To understand the process of fumaric acid production by microorganisms, the biochemistry and physiology of fumaric acid producers will be discussed first.

2.1. Microorganisms

2.1.1. Rhizopus

A number of microbial species are able to synthesize fumaric acid, but only a few produce significant quantities. Species that are considered to be of industrial importance to the production of fumaric acid belong to the genus *Rhizopus*. After decades of research, *R. nigricans*, *R. formosa*, *R. arrhizus* and *R. oryzae* have been unbeatable in their ability to efficiently produce fumaric acid under aerobic or anaerobic conditions (Carta et al., 1999; Foster and Davis, 1948; Liao et al., 2007; Rhodes et al., 1959). Table 2 summarizes the fumaric acid production abilities of *Rhizopus* spp. In the 1970s and 1980s, *R. arrhizus* was the main producer with the highest product concentration (121 g/L), but this species had a low yield (0.37 g/g) (Ling and Ng, 1989). Additionally, *R. arrhizus* requires rich nutrients, which resulted in increased material costs. In comparison, the nutrients required for *R. oryzae* are simple, allowing for lower material costs. Thus, after the 1990s, an *R. oryzae* strain was used as the main producer of fumaric acid and achieved the highest productivity of 4.25 g/L/h when grown in a rotary reactor (Cao et al., 1996). However, the fermentation process with the rotary reactor proved too complicated for industrial applications, and no further reports of this process are available.

It should be noted that not all strains of *Rhizopus oryzae* are able to produce fumaric acid. Abe et al. (2003) classified the strains of *R. oryzae* into two types: type I only formed fumaric acid with little or no lactic acid production, while type II formed lactic acid with little or no fumaric acid production. Saito et al. (2004) further showed that type I strains contain two lactate dehydrogenase genes, ldhA and ldhB, while type II strains only possess ldhB. After metabolic analysis, Oda et al. (2003) found that type I strains exhibited higher amounts of unsaturated fatty acids, and the proportions of palmitic and γ-linolenic acids were markedly different from type II. Moreover, based on data from rDNA ITS, ldhB, EF-1α and actin, as well as genomic AFLP, Abe et al. (2007) demonstrated that the fumaric acid producers may not belong to *R. oryzae* species, and they reclassified the fumaric acid producers as *R. delemar* while the lactic acid producers remained classified as *R. oryzae*. However, to avoid confusion, the following discussion will still designate fumaric acid producers as *R. oryzae*.

2.1.2. Genetically engineered strains

The construction of genetically engineered strains has also been considered as a mechanism for fumaric acid production. Kaclíková et al. (1992) constructed a mutant of *Saccharomyces cerevisiae* that