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Mini review

# Production of lactic acid from renewable materials by *Rhizopus* fungi

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**Abstract**

Lactic acid is a commonly occurring organic acid, which is valuable due to its wide use in food and food-related industries, and its potential for the production of biodegradable and biocompatible polylactate polymers. Lactic acid can be produced from renewable materials using various fungal species of the *Rhizopus* genus, which have advantages compared to the bacteria, including their amylolytic characteristics, low nutrient requirements and valuable fermentation by-product—fungal biomass. This paper reviews recent research in process engineering, metabolic and enzymetic mechanisms, and molecular biotechnology associated with lactic acid production by the *Rhizopus* fungi to get a better understanding of biochemical activities. The major process components: renewable materials, bioreactor systems and process modeling are reviewed. The role of key bioprocess parameters, such as nutrient composition, pH and growth morphology, involved in the production of lactic acid is discussed in detail. In addition, recent advances in simultaneous saccharification and fermentation, molecular genetic approaches, and enzymetic and metabolic pathways involved in the production of lactic acid by fungal strains are discussed.


*Keywords:* Lactic acid; Rhizopus species; Renewable materials; Simultaneous saccharification and fermentation; Starch; Enzymetic and metabolic pathway; Bioreactors; Immobilization; Lactate dehydrogenase

## Contents



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## 1. Introduction

Lactic acid (2-hydroxypropionic, $CH_3CHOHCOOH$) is the most widely utilized organic acid in the food, pharmaceutical, cosmetics and chemical industries. Its production is currently attracting a great deal of research and development. A review published in 1995 stated that 85% of lactic acid in the USA was used in food and food-related applications, and an emerging application was its use for production of biodegradable and biocompatible polylactate polymers, which provided an environmental friendly alternative to biodegradable plastics derived from petrochemical materials [1]. With the development and commercialization of these biopolymers, their use has increased considerably, and 20–30% of the 120,000-tonnes global production of lactic acid is estimated to be used in these new applications in 2005 [2].

Lactic acid exists naturally in two optical isomers: D(−)-lactic acid and L(+)-lactic acid. Since elevated levels of the D-isomer are harmful to humans [3], L(+)-lactic acid is the preferred isomer for food-related and pharmaceutical industries. Lactic acid can be produced commercially by either chemical synthesis or fermentation. The chemical synthesis results in a racemic mixture of the two isomers, while the fermentation process can yield an optically pure form of lactic acid or racemate, depending on microorganisms, substrates and fermentation conditions employed in the production process [4–6]. Renewable resources including sugars, starch and lignocellulose are abundant substrates for fermentative production. The most commonly used synthetic method for chemical production of lactic acid is the hydrolysis of lactonitrile derived from acetaldehyde and hydrogen cyanide, which are produced by petrochemical process. Therefore, chemical synthesis may be limited due to a shortage of the naturally available raw materials in the future.

Lactic acid can be produced using bacteria and fungi. Lactic acid producing bacteria (LAB) have received wide interest because of their high growth rate and product yield. However, LAB have complex nutrient requirements because of their limited ability to synthesize B-vitamins and amino acids [7], making supplementation of sufficient nutrients such as yeast extracts to media is necessary. This expensive downstream process increases the overall cost of production of lactic acid using lactic acid producing bacteria.

Fungal *Rhizopus* species have attracted a great interest, and have been recognized as suitable candidates for lactic acid production. Unlike the LAB, lactic acid producing *Rhizopus* strains generate L-lactic acid as a sole isomer of lactic acid [6,8–10]. The production of L-lactic acid using a surface culture of *Rhizopus* was reported in 1911 [11]. An efficient submerged fermentation using fungal species for the production of L-lactic acid was first reported in 1936 [12]. However, an increased research interest has been given to lactic acid fermentation by fungal species in recent decades. *Rhizopus* strains grow better under nitrogen-limited environments than the lactic acid producing bacteria [9,10,13]. When starch-based materials are used as substrate, only small amounts of inorganic salts and inorganic nitrogen need for lactic acid production using *Rhizopus* fungi. Separation of the fungal biomass from fermentation broth is easy because of their filamentous or pellet forms, leading to a simple and cheap downstream process required. In addition, as a by-product from lactic acid production, fungal biomass from *Rhizopus* strains can be used in biosorption processes for purification of contaminated effluents [14,15], for fungal chitiosan production [16,17] and as additive in animal feeds to improve the feed quality [18].

This paper reviews recent research in process engineering, metabolic and enzymetic mechanisms, and molecular biotechnology associated with lactic acid production by a member of the *Rhizopus* genus, and consequently to get a better understanding of biochemical activities. The major process components, renewable materials, bioreactor systems and process modeling are reviewed. The role of key bioprocess parameters, such as nutrient composition, pH and growth morphology, in the production of lactic acid is discussed in detail. In addition, recent advances in simultaneous saccharification and fermentation (SSF), molecular genetic approaches, and enzymetic and metabolic pathways involved in the production of lactic acid by fungal strains are discussed.

## 2. Substrates for lactic acid production

Renewable raw materials, including molasses, starch (corn starch, wheat starch, potato starch) and lignocellulose (corn cobs and woody materials) can be used as a substrate for fermentation of lactic acid. Previous studies on lactic acid production by *Rhizopus* species mainly used glucose as a substrate [6,9,19–29]. The effect of different carbohydrates on L-lactic acid production was investigated by Yin et al. [6] and Bulut et al. [19]. In many cases, glucose was the preferred carbon source for L-lactic acid production by *Rhizopus* species, followed by starch material. However, as cheap and widely existing materials, raw starch and lignocellulose were recognized as cost effective carbon sources for lactic acid production. This is due to that the substrate cost is one of the major operational costs, representing 30–40% of total production costs [30].