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Review

An overview of biotechnological production of propionic acid: From upstream to downstream processes

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ABSTRACT

The increasing demand for propionic acid (PA) production and its wide applications in several industries, especially the food industry (as a preservative and satiety inducer), have led to studies on the low-cost biosynthesis of this acid. This paper gives an overview of the biotechnological aspects of PA production and introduces *Propionibacterium* as the most popular organism for PA production. Moreover, all process variables influencing the production yield, different simple and complex carbon sources, the metabolic pathway of production, engineered mutants with increased productivity, and modified tolerance against high concentrations of acid have been described. Furthermore, possible methods of extraction and analysis of this organic acid, several applied bioreactors, and different culture systems and substrates are introduced. It can be concluded that maximum biomass and PA production may be achieved using metabolically engineered microorganisms and analyzing the most significant factors influencing yield. To date, the maximum reported yield for PA production is 0.973 $g \cdot g^{-1}$, obtained from *Propionibacterium acidipropionici* in a three-electrode amperometric culture system in medium containing 0.4 mM cobalt sepulchrate. In addition, the best promising substrate for PA bioproduction may be achieved using glycerol as a carbon source in an extractive continuous fermentation. Simultaneous production of PA and vitamin B_{12} is suggested, and finally, the limitations of and strategies for competitive microbial production with respect to chemical process from an economical point of view are proposed and presented. Finally, some future trends for bioproduction of PA are suggested.

Contents

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1. Introduction

Generally, *Propionibacterium* is a gram-positive, nonmotile, catalase-positive, nonspore-forming, and rod-shaped anaerobic to aerotolerant bacterium [1]. It is an important starter microorganism in dairy products and is widely used in the production of Swiss cheese [2,3], propionic acid (PA) [4,5,6], and vitamin B_{12} [7,8,9]. PA is an important chemical intermediate in the synthesis of herbicides, cellulose plastics, fruit flavors, ester solvents, perfume bases, and butyl rubber to improve the process ability and scorching resistance [1,10, 11,12]. Table 1 shows the physical and chemical properties of PA.

Like other organic acids, in nondissociated form, PA can pass through the cell membrane into the cytoplasm and release protons because of the intracellular alkaline pH. The resultant pH gradient across the cell membrane influences nutrient transfer [13,14] and inhibits the growth of fungi, yeasts, and some bacteria [1]. PA and its calcium, sodium, and potassium salts are widely used as preservatives in feed and foods because they are "generally recognized as safe" food additives by the Food and Drug Administration [15,16].

Commercial production of PA has been reported by chemical synthesis from petroleum feedstock. The acid could also be produced by *Propionibacterium* and some other anaerobic bacteria, e.g., *Selenomonas*, *Clostridium*, *Veillonella,* and *Fusobacterium* spp. [1,17,18].

The application of conventional expensive systems of fermentation is limited because of the low concentration, yield, and productivity of the process. Therefore, the increased yields of PA produced from the fermentation of cheap industrial waste (e.g., glycerol) as substrate or renewable sources (e.g., molasses, bagasse) can be economically justified [19,20]. The major problem with a batch system of fermentation is the strong inhibitory effect of the final product on the production yield, slow growth of bacteria [21,22], and difficulty of extraction from the media [23,24]. Some processes, including multi-stage [25], cell immobilization [26], fed-batch [27,28,29], continuous culture [30], and extractive fermentation [6,31] systems, have been used to increase the yield of PA production.

In addition to these features, PA has a satiety-inducing effect on human diet by stimulating the release of peptide YY in the colon as an appetite suppressor [32,33,34,35,36]. Despite this interesting feature, no comprehensive reviews have been reported on the biosynthesis of PA.

Table 1
Chemical and physical properties of propionic acid.

Chemical and physical properties	
IUPAC name	Propanoic acid
Other names	Ethanecarboxylic acid, propionic acid
CAS number	79-09-4
Molecular formula	$C_3H_6O_2$
Molar mass	74.08 g/mol
Appearance	Colorless liquid
Odor	Slightly rancid
Melting point	-21°C
Boiling point	141°C
Density	0.99 g/cm3
Solubility in water	Miscible
Acidity (pKa)	4.87
Viscosity	10 mPa·s

In this study, different aspects of PA such as its chemical properties, applications, biochemical pathway, microbial biosynthesis, different reported microbial species, and carbon and nitrogen sources, as well as culture systems, bioreactors, analysis, methods of recovery, and simultaneous production of acid and vitamin B_{12} are reviewed.

2. Chemical properties of PA

PA is a colorless, organic corrosive liquid acid with a sharp and pungent odor [12]. This acid possesses physical properties that are between those of the slighter carboxylic, formic, and acetic acid and long-chain fatty acids. It represents the overall properties of carboxylic acids and forms amide, ester, anhydride, and chloride compounds [21]. It is miscible with water, and addition of salt leads to solving out of it from the water phase. It can react with alcohols, basis of esters, and organic salts [12,21].

3. PA production

PA can be produced by chemical (oxidation of propanol or propanal and hydrolysis of esters) and microbial/biotechnological methods [1]. Currently, PA is produced almost exclusively through petrochemical processes by the oxidation of propane or propionaldehyde as raw material [11], with an annual production capacity of ~400 million lbs in the US. As the crude oil values have exceeded $56/barrel (1 year forecast/2016 based on http://www.oil-price.net/?gclid=CIix8MaGg9ACFcYV0wodXBsCOQ), there has been an increasing trend in the biosynthesis of PA from renewable resources by culturing certain microorganisms, mainly *Propionibacterium* [37].

Despite some advantages of bioproduction, there are also a few limitations, which economically disadvantage fermentative processes when compared to chemical processes including fastidiousness of task, time-consumption (2 weeks in batch fermentation), end product inhibition, and expensive downstream processing of recovery and concentration [38].

However, there is an increasing trend in the application of PA as an important natural preservative. Therefore, fermentative production remains attractive because of high price of oil and petrochemical products and necessity of usage of renewable resources. By the application of cheap agroindustrial wastes and renewable feedstock, microbial production can commercially compete with chemical processes [5,11,14,19,25].

3.1. Microbial production of PA

PA can be produced by slowly growing gram-positive bacteria, e.g., *Propionibacterium*, and some gram-negative anaerobes, e.g., *Selenomonas ruminantium*, *Anaerovibrio lipolytica*, *Veillonella* spp., *Propionispira arboris*, and *Bacteroides fragilis* [38,39,40,41].

3.2. Process variables influencing acid production by Propionibacterium

Several factors influence PA fermentation including microorganism species, pH, temperature, carbon sources, inoculum size, time of fermentation, and nitrogen source type and concentration (Table 2).

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