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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 (p*Ka*)	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 water soluble, but addition of salt leads to its precipitation (salt break it away from water phase). It reacts with alcohols, esters basis, 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].

It is true that bioproduction has some advantages; it is not without its pitfalls and limitations. As when it is compared with chemical processes it could be regarded as an economically disadvantage fermentative process in terms of fastidiousness of task, time-consumption (2 weeks in batch fermentation), end product inhibition, 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).

Table 2
Production of propionic acid on several carbon sources by various microorganisms and culture systems.

Carbon source	Bacteria	Culture system	Time (h)	Propionic acid			P/A ratio	Reference
				Concentration $g \cdot L^{-1}$	Yield $g \cdot g^{-1}$	productivity $g \cdot L^{-1} \cdot h^{-1}$		
Flour hydrolysate	*P. acidipropionici*	Fed-batch	160	30	–	0.33	–	[56]
Glucose	*P. freudenreichii*	Fed-batch	160	52.5	0.66	–	–	[8]
Glycerol	*P. acidipropionici*	Fed-batch	240	44.62	–	0.20	–	[18]
Glycerol	*P. acidipropionici*	Fed-batch	2880 (4months)	~106	–	–	–	[81]
Lactate	*P. acidipropionici*	Fed-batch	2160 (3months)	~100	–	–	–	[14]
Glycerol	*P. zeae*	Batch	140	8.98	0.788	0.064	15.0:1	[12]
Lactate	*P. jensenii*	Batch	140	16.31	0.468	0.117	2.9:1	[12]
Sugarcane molasses	*P. zeae* (CCT 5329)	Batch	140	6.83	0.604	0.049	2.8:1	[12]
Lactate	*P. acidipropionici*	Batch	133	15.06	0.44	0.11	2.7:1	[1]
Glycerol	*P. acidipropionici*	Batch	133	6.77	0.72	0.05	100.0:1	[1]
Sugarcane molasses	*P. acidipropionici*	Batch	133	8.23	0.45	0.06	3.7:1	[1]
Cheese whey	*P. acidipropionici*	Batch	–	3.30	–	–	–	[89]
Glucose	*P. acidipropionici*	fibrous bed bioreactor	–	71.8	–	–	–	[57]
Glucose/lactate	*P. acidipropionici*	Fed-batch	40	~30	–	–	7.63:1	[45]
Lactose	*P. acidipropionici*	Continuous, in situ cell retention	192 (8 days)	–	0.402	0.90	–	[48]
Cheese whey	*P. acidipropionici*	Continuous with cell retention	120	–	0.70	0.98	–	[83]
Lactose	*P. acidipropionici*	Batch with extractive fermentation	1080 (1.5months)	75	-	~1	–	[6]
Glucose/glycerol	*P. shermanii*	Batch		~9	–	–	–	[49]
Glucose	*P. shermanii*	Batch	72	12.5	–	–	–	[47]
Glucose	*P. acidipropionici*	Semi-continuous	192 (8 days)	47	0.45	0.37	3.6	[19]
Glucose	*P. beijingense*	Batch	–	11.32	–	–	–	[90]

3.2.1. Microorganism species

PA can be produced through fermentation by growing certain bacteria including *S. ruminantium* [42], *Propionibacterium acidipropionici* [18], and *Propionibacterium freudenreichii* [43]. *P. acidipropionici* is "generally recognized as safe" and has been used in the dairy industry for cheese production [44].

3.2.1.1. Propionibacterium as the most promising producer. *Propionibacterium* are gram-positive, catalase-positive, nonmotile, rod-shaped, and nonspore-forming facultative anaerobes that can grow on several types of carbon sources. The optimum temperature and pH for cell growth of *Propionibacterium* are 30–37°C and 6–7, respectively. At pH less than 4.5, the growth will stop, stopping acid formation [12,25].

"Cutaneous" Propionibacteria mostly cause diseases, and the most important member is *Propionibacterium acnes,* which causes acne and skin diseases. "Dairy" Propionibacteria are broadly used in the production of Swiss cheese [3,12], PA [1,45,46], and vitamin B_{12} [7,8,12]. Propionibacteria are also used as a probiotic and cheese starters in silage processing [47]. Several species including *Propionibacterium zeae*, *Propionibacterium thoenii*, *P. freudenreichii*, *Propionibacterium shermanii*, *P. acidipropionici*, *Propionibacterium jensenii,* and *Propionibacterium beijingense* have been studied from biotechnological aspects. Among them, *P. acidipropionici* [12,48,49], *P. shermanii* [12,47,50,51], and *P. freudenreichii* spp. *shermanii* [49,52,53] have been mostly used because of their ability to produce PA. For the industrial production of PA, the most commonly strain used is *P. acidipropionici* [12,19,45]. According to Fitz equation,

$$\begin{aligned} &3 \text{ lactic acid} \rightarrow 2 \text{ propionic acid} + 1 \text{ acetic acid} + 1\ CO_2 + 1\ H_2O \\ &1.5 \text{ glucose} \rightarrow 2 \text{ propionic acid} + 1 \text{ acetic acid} + 1\ CO_2 + 1\ H_2O \end{aligned} \quad (1)$$

Theoretically extreme outputs are 54.8% (w/w) PA and 77% aggregate acids. The production of PA is coupled with the formation of

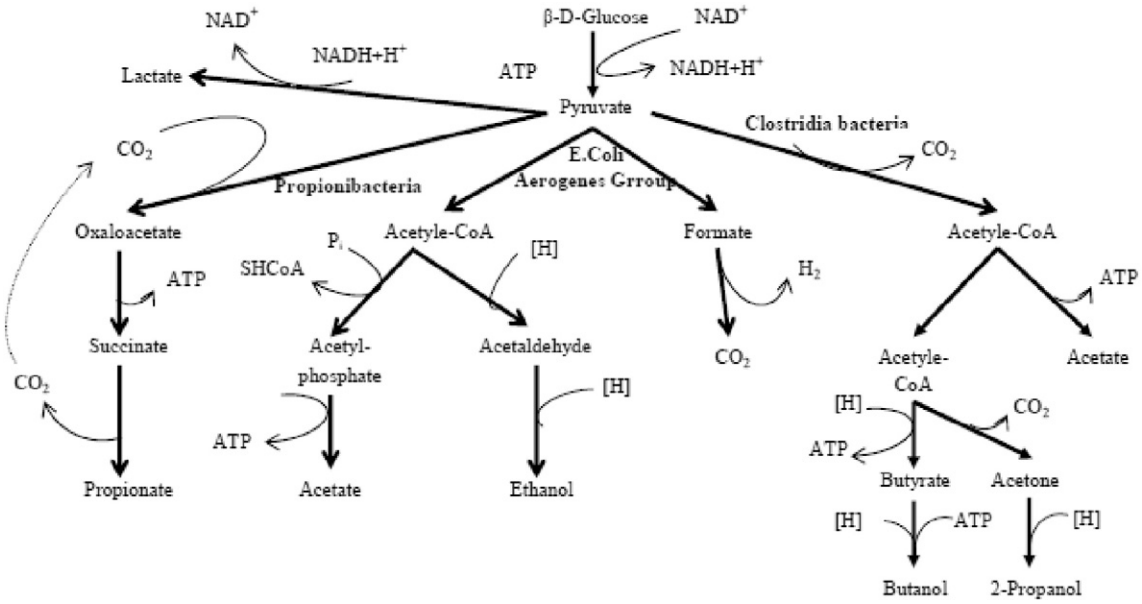

Fig. 1. Different pathways of propionic acid production.

acetate and preservation of hydrogen and redox steadiness [11]. There are two pathways of PA formation: dicarboxylic and acrylic acid pathway. The dicarboxylic acid pathway is more general, while the acrylic trail is limited to few types of bacteria (*Clostridium propionicum*, *Bacteroides ruminicola, and Megasphaera elsdenii*). PA-producing bacteria mostly prefer to use lactate than glucose as substrate [11,12]. Fig. 1 indicates the different pathways of PA production.

Coral [12] studied eight strains of *Propionibacterium* for the production of PA using lactate, glycerol, and sugarcane molasses as carbon sources. The results showed that *P. zeae* (DSM 20274) was the most adapted *Propionibacterium* for growth on glycerol, reaching 8.98 $g \cdot L^{-1}$ PA and 2.86 $g \cdot L^{-1}$ dry biomass, while *Propionibacterium arabinosum* (ATCC 4965) and *P. acidipropionici* showed the best results when sugarcane molasses was used as carbon substrate at a bench-scale fermentation [12].

3.2.1.2. Co-culture. Conventional PA fermentation systems were typically monocultures. Although mixed cultures have been reported as a suitable alternative, they have not been well improved, due to low productivity, up until now [54,55].

Sabra et al. [56] showed the effective production of PA by co-culturing *Lactobacillus zeae* and *Veillonella criceti*. To decrease substrate and total cost for possible application in food industry, flour hydrolysate was used as the carbon source. Using the fed-batch method and without the addition of yeast extract, a maximum PA concentration of 30 $g \cdot L^{-1}$ and productivity of 0.33 $g \cdot L^{-1} \cdot h^{-1}$ was achieved.

3.2.1.3. Metabolic engineering. Metabolically engineered mutants of *Propionibacterium* resulted in increased productivity and yield of PA and modified tolerance against high concentrations of acid [57]. Immobilized adapted cells of *P. acidipropionici* produce a high concentration of 72 $g \cdot L^{-1}$ PA from glucose in a fibrous-bed bioreactor [14,58]. Moreover, acid-tolerant *P. acidipropionici* (ATCC 4875) produced PA using glycerol as substrate at a concentration of 106 $g \cdot L^{-1}$, which was 2.5 times more than the maximum concentration of acid produced from glucose (approximately 42 $g \cdot L^{-1}$) [14]. A scaled-up process of PA production from glycerol by acid-tolerant *P. acidipropionici* (CGMCC 1.2230) was studied in a 7-L batch bioreactor. The bacterium was adapted to 20 $g \cdot L^{-1}$ concentration of acid by serial transfers into media containing increasing concentrations of acid [59].

Zhuge et al. [60] studied fed-batch fermentation of PA with metabolically engineered *P. jensenii* (pZGX04-gldA). In this study, a two-stage pH control strategy in a 3-L fermentor with a constant feeding rate was reported, and a maximum PA production of 34.62 g/L was obtained. The final concentration and productivity reached 37.26 $g \cdot L^{-1}$ and 0.163 $g \cdot L^{-1} \cdot h^{-1}$, respectively. Then, they investigated a three-stage oxidoreduction potential control strategy to improve PA production by using engineered *P. jensenii* ATCC 4868 (pZGX04-gldA) in a 3-L bioreactor [60]. Oxidoreduction potential was controlled at -200 mV for 36 h (to accelerate cell growth) and shifted to -300 mV between 36 and 156 h (to maintain cell growth). Finally, it was shifted to -400 mV after 156 h to maximize PA accumulation. PA production reached 27.31 $g \cdot L^{-1}$, and further improved to 39.53 $g \cdot L^{-1}$ after integrating the oxidoreduction potential shift strategy in a fed-batch culture method.

Wei et al. [61] successfully introduced xyl operon genes from *P. acidipropionici* into *P. shermanii* to enable the latter to use both xylose and glucose simultaneously without glucose-mediated carbon catabolite repression (CCR), allowing the use of this organism in biorefineries using lignocellulosic biomass feedstock. *P. freudenreichii* ssp. *shermanii* cannot use xylose as a fermentation substrate. For industrial applications, this bacteria needs the ability to consume a wide range of sugars including xylose as it is the next abundant sugar in lignocellulosic biomass.

Pyruvate carboxylase, methylmalonyl-CoA decarboxylase, and methylmalonyl-CoA carboxyl transferase are biotin-dependent carboxylases that were over-expressed in *P. freudenreichii* ssp. *shermanii* DSM 4902 to study their effects on PA fermentation in serum bottles containing glucose and glycerol/glucose mixture as the carbon source [62]. Compared to the wild type, mutants overexpressing methylmalonyl-CoA carboxyl transferase and methylmalonyl-CoA decarboxylase showed a significantly different metabolic flux distribution favoring more PA production (compared to acetic and succinic acids), with significantly increased yield (up to 14% increase) and productivity (up to 17% increase), especially in the cofermentation of glycerol and glucose. In addition, the mutant overexpressing pyruvate carboxylase grew slower and produced more succinate and less propionate (up to 12% decrease in productivity) [62].

3.2.2. pH

Propionibacterium are very susceptible to pH. Therefore, pH control is important as growth in inhibited in pH less than 5.0 [1]. pH has a great effect on the lag phase, production rate, and yield (cells and product per substrate). The ratio of propionic to acetic acid was significantly influenced by pH [23].

Seshadri and Mukhopadhyay [23] reported that lower pH improves the ratio of propionic to acetic acid concentration (5:1); however, the PA productivity was low (0.11 $g \cdot L^{-1} \cdot h^{-1}$). In pH greater than 6 (up to 7.5), lower ratios of PA to acetic acid concentration (2.5:1) was achieved, but the productivity of PA increased (0.2 $g \cdot L^{-1} \cdot h^{-1}$). Lactate as a carbon source has strong benefits over glycerol and sugarcane molasses when tight pH control cannot be applied [1]. pH variation increased with the usage of glycerol and sugarcane molasses when compared to the usage of lactate [1,4].

By using the two-stage pH control strategy (pH of 6.5 for 48 h and then at 6.0), PA concentration reached to a maximum of 19.21 $g \cdot L^{-1}$, while it was 14.58 $g \cdot L^{-1}$ with a constant pH [63].

3.2.3. Temperature

Temperature is one of the most important factors influencing the yield of PA biosynthesis. Seshadri and Mukhopadhyay [23] reported that PA production was reduced when temperatures increased from 30°C to 37°C. Quesada-Chanto et al. [64] stated that 37°C is the optimal temperature for PA production. However, Coral et al. [1] observed that PA and biomass production were superior at 30°C than at 36°C. Farhadi et al. [65] observed that when the temperature was increased from 30 to 35 and 40°C, PA and acetic acid production decreased, while cell biomass increased.

3.2.4. Carbon sources

Propionibacteria are capable of using different carbon sources including glucose [12,45,49,66], fructose [67,68], maltose [69], sucrose [12,64], xylose [12,46], lactose [12,70,71], glycerol [12,14,49,72], molasses [12,28], and lactate [12,45,66].

However, low productivity and concentration inhibit the widespread application of expensive conventional fermentation systems [12]. Therefore, more studies were conducted to investigate the application of cheap agroindustrial wastes or renewable sources (such as glycerol and sugarcane molasses) [1,38,73] or low-cost substrates (such as whey, flour hydrolysates, steep water, maize gluten, wood pulp waste liquor, sulfite waste liquors, and lignocelluloses) and develop a more proficient downstream processing [1,53,65,74,75,76].

Rapid development of biodiesel production, as a substitute for petroleum diesel, leads to excess synthesis of crude glycerol. Therefore, crude glycerol is an economically possible substance for industrial applications [77]. Production of PA from glycerol, compared to production from glucose, results in increased production yield but

decreased acetic acid formation. The maximum PA yield was obtained, and the PA to acetic acid (P/A) ratio was 100:1 [1,14,18,49]. Therefore, recovery and downstream processing are easier with the use of glycerol than lactate and molasses. Moreover, there was a 70% decrease in the total cost of produced acid from agroindustrial waste of biodiesel [1]. Numerous studies have revealed that glycerol can be used for the production of more condensed metabolites [78,79,80] but can lead to redox imbalance in metabolism, causing reduced cell evolution and yield, when used as the solitary carbon source in fermentation [14,49].

Coral et al. [1] showed the effect of different carbon sources (lactate, sugarcane molasses, and glycerol) on PA fermentation. The results showed that the maximum final concentration and productivity of PA was obtained from lactate. In fact, the production of PA from lactate was faster than from molasses because it does not need to be metabolized through the glycolytic pathway. Another clear advantage of using lactate over sugarcane molasses and glycerol is that it does not require constant pH control because of limited pH variation. The maximum cell growth rate, productivity, yield, and concentration of produced biomass were obtained when sugarcane molasses were used as substrate (this feature is desired for the simultaneous production of PA and vitamin B_{12}).

3.2.4.1. Mixed substrates. Jerusalem artichoke-based medium containing 10 $g \cdot L^{-1}$ yeast extract was also used for PA production by *P. acidipropionici,* resulting in 40 $g \cdot L^{-1}$ PA, with a productivity of 0.26 $g.L^{-1}$ h^{-1} [76]. The production of 29.2 $g \cdot L^{-1}$ PA by using a mixture of glycerol and glucose was reported by Liu et al. [73]; however, the output was rather small (0.15 $g \cdot L^{-1} \cdot h^{-1}$) and the medium used was costly.

With the use of mixture of glycerol and glucose [73], the concentration and productivity of PA decreased to 29.2 $g \cdot L^{-1}$ and 0.15 $g \cdot L^{-1}$ h^{-1}, respectively. Co-fermentation of glycerol and glucose at a suitable mass proportion provided a yield of 0.54–0.65 $g \cdot g^{-1}$ and productivity of 0.18–0.23 $g \cdot L^{-1} \cdot h^{-1}$, with high product discrimination (ratio of PA/Acetic acid: 14; PA/mass). The carbon flux distribution in the co-fermentation was influenced by the ratio of glycerol to glucose. Moreover, co-fermentation using cassava bagasse hydrolysate and crude glycerol in a fibrous-bed bioreactor was reported, giving an effectual way for the commercial production of bio-based PA [73].

3.2.4.2. Complex sources. Liu et al. [16] reported the production of PA from hemicelluloses by *P. acidipropionici* ATCC 4875 in biorefinery processes. Using xylose (the predominant sugar in hemicelluloses) as the sole carbon source, a final PA concentration of 53.2 $g \cdot L^{-1}$ was obtained through fed-batch fermentation. However, for this strain, hemicellulose hydrolysate (corncob molasses, a waste by-product in xylitol production) was used as an excellent carbon source for efficient PA production to obtain a final concentration of 71.8 $g \cdot L^{-1}$ and productivity of 0.28 $g \cdot L^{-1} \cdot h^{-1}$.

As previously noted, xylose was efficiently consumed in the presence of glucose by the engineered mutant *P. freudenreichii* ssp. *shermanii* [61]. The production of PA on several carbon sources, various microorganisms, and culture systems are shown in Table 2.

3.2.5. Time of fermentation

During the fermentation process, the PA concentration reached the maximum value but upon further fermentation, the acid production decreases. Extending the fermentation time reduces the productivity because of the accumulation of inhibitory metabolites. Therefore, it is very critical to select the optimum time for the fermentation process. PA production has been reported for different times such as, 48, 96, 120, 144, and 168 h [1,81,82], but no comprehensive study about optimum time has been reported. Importance of this factor is directly related to the productivity of the acid.

3.2.6. Nitrogen sources

The type and concentration of nitrogen source also affect the microbial production of PA significantly. Yeast extract, peptone, and corn steep liquor are good sources that can be used by *Propionibacterium* spp. effectively [82]. Nitrogen sources in the concentration range of 540 $g \cdot L^{-1}$ have been used in different studies for PA fermentation, and the most commonly applied concentrations were 5 and 10 $g \cdot L^{-1}$ [1,12,14,18]. More investigation in the future is required to evaluate the dependency of yield and costs on different types of nitrogen sources.

3.2.7. Culture systems

PA production has been achieved by applying various culture systems such as batch, fed-batch, and continuous fermentation [1,83]. The advantages and disadvantages of each method have been studied with the aim of high production of PA at low costs.

Batch-type fermentations are the most commonly used system to produce PA where the production process takes 2 weeks for completion [11]. The main obstacle in the batch fermentation process is the slow growth of bacteria and the vigorous end product inhibition [48]. Conventional bioprocess of PA production suffers from low concentration (<40 $g \cdot L^{-1}$), low yield (<0.5 $g \cdot g^{-1}$), and low productivity (<1 $g \cdot L^{-1} \cdot h^{-1}$). However, batch fermentation was applied in most of the reported case because it allows easy control of the process.

Fed-batch fermentations with and without extractive membranes have also been used for increasing the production rates and overall yields of fermentation [42,46,48]. Addition of sugar [48] and/or other carbon sources [45,48] at frequent intervals was the most commonly used feeding strategy. Coral et al. [1] first reported that such a system can be used for the simultaneous production of vitamin B_{12} and PA.

Improved PA productivity is necessary to make the microbial production process economically viable. To our knowledge, few studies have been conducted on PA production using continuous fermentation systems, although they are known to increase productivity. Gupta et al. [83] studied continuous PA production from cheese whey using *in situ* spin filter. They reported a PA production yield of 51% and 54% (based on the amount of consumed lactose) in batch and continuous systems, respectively. The yield of PA in the continuous fermentation system with cell retention even reached to 70% w/w. Volumetric productivity in batch and continuous conditions was reported as 0.312 and 0.715 $g \cdot L^{-1} \cdot h^{-1}$, respectively. Continuous fermentation with cell retention resulted in the highest volumetric productivity value of 0.98 $g \cdot L^{-1} \cdot h^{-1}$ [83].

3.2.7.1. Extractive fermentation. Extractive fermentation can circumvent the problem of the inhibitory effect of acidic end products. This method can also provide a better pH control, resulting in higher production rates with less bioproducts. Consequently, the downstream recovery can be performed in a much simple way, with a lesser purification cost [5,6,84]. The final products are mostly PA and acetic acid that can be removed continuously by solvent extraction [6]. However, some authors also noted certain disadvantages because of the difficulty in the selection of a solvent and this method's high dependency on pH and, consequently, high costs of operation [4,13,85].

An extractive fermentation process was developed to produce propionate from lactose by *P. acidipropionici,* which was immobilized in a spirally wound fibrous matrix packed in the bioreactor (a novel packed-bed immobilized cell bioreactor that was used for long-term continuous PA fermentation). PA is the main product of lactose fermentation, with a strong end product inhibition. In addition, acetic acid and carbon dioxide are by-products of this process [4].

The productivity of the extractive fermentation (~1 $g \cdot L^{-1} \cdot h^{-1}$ or five-fold increase) was much more than that of the conventional batch process. Moreover, in extractive fermentation, an increased propionate yield (up to 0.66 $g \cdot g^{-1}$ with 20% increase), a final product

concentration of 75 $g \cdot L^{-1}$, and increased product purity (~90%) were achieved. Furthermore, the production of both acetate and succinate was significantly reduced. The improved fermentation efficacy is because of less product inhibition and a possible metabolic pathway shift to favor more PA production and less acetic and succinic acid production [6].

3.3. Simultaneous production of PA and Vitamin B_{12}

Industrial-scale production of vitamin B_{12} is possible in both batch and fed-batch fermentations. Simultaneous production of PA and vitamin B_{12} was reported by *P. acidipropionici* on molasses and sugar in a continuous process. However, other studies have not reported the simultaneous production of PA and vitamin B_{12}. Quesada-Chanto et al. [64] investigated the microbial production of PA and vitamin B_{12}. The maximum amount of produced PA and vitamin B_{12} was reported as 17.0 $g \cdot L^{-1}$ and 34.8 $mg \cdot L^{-1}$, respectively.

In a study by Wang et al. [8], by applying fed-batch fermentation in an expanded bed adsorption bioreactor and using molasses or sugar as carbon sources, 52.5 $g \cdot L^{-1}$ PA and 43.04 $mg \cdot L^{-1}$ vitamin B_{12} were obtained. Simultaneous production of PA and vitamin B_{12} by several sub-species of *Propionibacterium* on different carbon sources are shown in Table 3.

4. Bioreactors

Different bioreactors have been reported for PA production such as cell recycle, immobilized cell, and fibrous-bed bioreactors; calcium-alginate beads; and extractive fermentation. The amount of PA production according to the type of bioreactor has been listed in Table 4.

5. Downstream processing

There are several methods for the extraction of organic acids that are produced in aqueous solutions. These methods include liquid extraction, reverse osmosis, electrodialysis, liquid surfactant membrane extraction, anion exchange, precipitation, adsorption, and reactive extraction. Calcium hydroxide precipitation method is a conventional method for the recovery of PA from fermentation broth. However, it is expensive and hostile to the environment because of the use of solvents and sulfuric acid and the production of a large quantity of calcium sulfate sludge as solid waste. Therefore, there is a strong necessity to search for new methods for the recovery of PA [86].

Reactive extraction has been introduced as a promising method for the extraction of carboxylic and hydroxycarboxylic acids. Some of the advantages of this method include increased concentration, increased productivity, easy control of pH without requiring base addition, decreased process wastes, and decreased production costs. A continuous step for production and recovery leads to reduced time of downstream processing and less recovery costs [87,88].

6. Applications of PA

PA, which occurs naturally in grains, apples, cheese, and strawberries and human sweat, is used as a solvent (alkyl propionate esters) [11,25] and an intermediate in the manufacturing of several chemicals; some examples are as follows:

I. Herbicides (for the synthesis of sodium 2, 2-dichloropropionate) [11];
II. Chemical intermediates (for production of plastics, plasticizers, textile and rubber auxiliaries, and dye intermediates) [37];
III. Artificial flavors and fragrances (as a precursor for the chemical synthesis of propionic ether and benzyl propionate) [37];
IV. Pharmaceuticals (used for the synthesis of propionic anhydride and chloropropionic acid);
V. Cellulose acetate propionate (CAP) (as a precursor for the synthesis of CAP);
VI. Preservative for food (used as food preservative to prevent molding in foods like bread and cake);
VII. Animal feed and grain (salts of PA are primarily used for animal feed preservation, including hay, silage, and grains, because PA can inhibit *Aspergillus flavus*, aerobic *Bacillus* spp., *Salmonella* spp., and yeast) [1,34].

Table 5 shows the most common industrial applications of PA.

7. Conclusion

In this paper, some of the aspects of biotechnological production of PA in submerged systems by *Propionibacterium* as a common producer were discussed. In addition, the different ways in which the production yield depends on the substrate, culture conditions, kind and scale of bioreactors, and microorganism species were reviewed. It can be concluded that *P. acidipropionici* may potentially be used for the production of PA using glycerol media as a cheap agroindustrial waste. Moreover, simultaneous production of PA and vitamin B_{12} by *P. freudenreichii* from glucose was mentioned as an interesting alternative. A review of the reports shows that appropriate pre-adaptation of the microorganisms and applications of metabolically engineered mutants may lead to increased production yields of biomass and PA. The most significant factors influencing PA production in submerged fermentation are temperature and pH, which directly influence high yields.

Although there is a way to go before industrial-scale biosynthesis of PA is realized, a mutant strain of *P. acidipropionici* (ATCC 4875) with ack gene (encoding acetate kinase) knockout (ACK-test) has already been used in a 5-L bioreactor, and a product concentration of 106 $g \cdot L^{-1}$ was attained. To date, the maximum reported yield for PA production is 0.973 $g \cdot g^{-1}$, which was obtained from the cultivation of *P. acidipropionici* in a three-electrode amperometric culture system in medium containing 0.4 mM cobalt sepulchrate.

Table 3
Simultaneous production of propionic acid and vitamin B_{12} by several subspecies of *Propionibacterium* spp. on different carbon sources. (Modified from Quesada-Chanto et al. [64]).

Organism	Substrate	Type of fermentation	Propionic acid ($g \cdot L^{-1}$)	Vit. B_{12} ($g \cdot L^{-1}$)	References
P. acidipripionici ATCC4975	Whey	Continuous	25.0	_	[91]
P. acidipropionici ATCC4875	Whey	Continuous	8.5	_	[92]
P. acidipropionici ATCC4875	Whey	Continuous	7.0	_	[30]
P. acidipropionici ATCC4875	Whey	Continuous	16.7	_	[30]
P. acidipropionici ATCC25562	Whey	Continuous	24.2	_	[93]
P. acidipropionici DSM 8250	Sugar	Continuous	30	33	[64]
P. acidipropionici DSM 8250	Sugar	Continuous	16.2	16.8	[64]
P. acidipropionici DSM 8250	Blackstrap molasses	Continuous	17.7	28.8	[64]
			17.0	34.8	[64]
P. freudenreichii CICC 10019	Glucose	Fed-batch	52.5	43.04	[8]

Table 4
Amount of propionic acid production according to the type of bioreactor.
(Modified from Coral, [12]).

Fermentative process	Microorganism	Amount of propionic acid production			Carbon sources	Reference
		Concentr. $g \cdot L^{-1}$	Yield $g \cdot g^{-1}$	Productivity $g \cdot L^{-1} \cdot h^{-1}$		
Fibrous-bed bioreactor (repeated batch fermentations)	*P. freudenreichii*	–	0.54–0.65	0.18–0.23	Glycerol/glucose	[38]
High-cell-density sequential batches with cell recycle	*P. acidipropionici*	50.8	–	–	Glycerol/ potato juice	[94]
Immobilized cell in fibrous-bed bioreactor (fed-batch)	Adapted acid-tolerant *P. acidipropionici*	51.2	–	–	Glucose	[59]
Anion exchanger-based in situ product recovery (fed-batch)	*P. freudenreichii* (CICC 10019)	62.5	0.78	–	Glucose	[8]
In situ product removal of expanded bed adsorption bioreactor (fed-batch)	*P. freudenreichii* (CICC 10019)	52.5	0.66	–	Glucose	[8]
Multi-point fibrous-bed bioreactor (fed-batch)	*P. freudenreichii*	67.05	–	0.14	Glucose	[63]
Cell immobilization fibrous-bed bioreactor (batch)	*P. acidipropionici*	–	0.58	2012	Corn meal §	[75]
Immobilized cell in a stirred tank fermentor	*P. acidipropionici*	18.61	–	0.31	Lactose	[46]
Calcium-alginate beads(repeated-batch)	*Propionibacterium*	34	0.45	–	glucose	[66]
Extractive fermentation (hollow-fiber member) (fed-batch)	*P. acidipropionici*	75	0.66	1	Lactose	[6]
Cell immobilization fibrous-bed bioreactor (recycle-batch)	*P. acidipropionici*	65	0.5	0.22–0.47	lactose permeate	[24]
Three-electrode amperometric culture system (batch)	*P. freudenreichii*	–	0.973	–	Glucose	[95]
Cell recycle fermentation	*P. acidipropionici*	–	–	2.7	Xylose	[96]
Stirred-tank reactor with ultrafiltration recycles (continuous)	*P. acidipropionici*	–	–	14.3	sweet-whey permeate	[91]
Calcium-alginate beads (fed-batch) (continuous)	*P. acidipropionici* (P200910) (P200910)	57 –	– –	0.3 0.96	Semi-defined medium	[84]

§ Corn meal hydrolyzed.

By reviewing the several reports on the production of this short-chain fatty acid on a wide range of carbon sources in different culture systems and conditions, it can be concluded that the most promising method of PA bioproduction may be achieved by growing *P. acidipropionici* on glycerol as a carbon source in an extractive continuous fermentation.

The best raw material that is advantageous in terms of availability and costs is glycerol. It may decrease the number of downstream steps and increase the final product quality because of the absence of acetic acid as a by-product. Moreover, *in situ* product recovery process for the simultaneous optimized production of PA/vitamin B_{12} in a fibrous-bed bioreactor or expanded bed adsorption bioreactor can be a future trend (expanded bed adsorption, a recently developed technology in separation field, has some potential advantages when used for *in situ* product removal). Research on some inherent satiety-inducing properties, such as the infusion of short-chain fatty acids into the colon, stimulation of the release of peptide YY, and induction of appetite-suppressing effect, would further support this field of research.

Table 5
The most common industrial applications of PA.

The industrial use	The remarks	References
Herbicides	For the synthesis of sodium 2, 2-dichloropropionate	[11,97]
Chemical intermediates	for production of plastics, plasticizers, textile and rubber auxiliaries, and dye intermediates	[37]
Artificial flavors and fragrances	as a precursor for the chemical synthesis of propionic ether and benzyl propionate	[37]
Pharmaceuticals	for the synthesis of propionic anhydride and chloropropionic acid	[37]
Cellulose acetate propionate (CAP)	as a precursor for the synthesis of CAP	[1,62]
Preservatives for food	used as preservative in food industries to prevent the foods such as bread and cake from molding	[1,42,62]
Animal feed and grain	salts of PA are primarily used for animal feed preservation, including hay, silage, and grains because of PA can inhibit *Aspergillus flavus*, aerobic Bacillus spp., Salmonella spp., and yeast	[1,37]

Conflict of interest

The authors confirm that this article content has no conflicts or declaration of interest.

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