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Review

Microbial Production of Malic Acid from Biofuel-Related Coproducts and Biomass

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Abstract: The dicarboxylic acid malic acid synthesized as part of the tricarboxylic acid cycle can be produced in excess by certain microorganisms. Although malic acid is produced industrially to a lesser extent than citric acid, malic acid has industrial applications in foods and pharmaceuticals as an acidulant among other uses. Only recently has the production of this organic acid from coproducts of industrial bioprocessing been investigated. It has been shown that malic acid can be synthesized by microbes from coproducts generated during biofuel production. More specifically, malic acid has been shown to be synthesized by species of the fungus *Aspergillus* on thin stillage, a coproduct from corn-based ethanol production, and on crude glycerol, a coproduct from biodiesel production. In addition, the fungus *Ustilago trichophora* has also been shown to produce malic acid from crude glycerol. With respect to bacteria, a strain of the thermophilic actinobacterium *Thermobifida fusca* has been shown to produce malic acid from cellulose and treated lignocellulosic biomass. An alternate method of producing malic acid is to use agricultural biomass converted to syngas or biooil as a substrate for fungal bioconversion. Production of poly(β-L-malic acid) by strains of *Aureobasidium pullulans* from agricultural biomass has been reported where the polymalic acid is subsequently hydrolyzed to malic acid. This review examines applications of malic acid, metabolic pathways that synthesize malic acid and microbial malic acid production from biofuel-related coproducts, lignocellulosic biomass and poly(β-L-malic acid).

Keywords: malic acid; biomass; polymalic acid; plant hydrolysates; *Aureobasidium*; *Aspergillus*; *Thermobifida*; *Rhizopus*

1. Introduction

Malic acid is considered as a "building block" chemical for the production of biodegradable polymers [1,2]. Originally, malic acid was extracted from eggshells, fruits and apple juice but the cost of isolating malic acid in that fashion was very expensive since it was labor intensive [2]. The commercial use of malic acid is varied [1–3]. In foods and beverages, it has been used as an acidulant [1,2]. As an acidulant, malic acid has a less bitter taste than citric acid [1,2]. Malic acid can also enhance the flavor of foods [1,2]. Other commercial applications of malic acid are in metal cleaning, textile finishing, pharmaceuticals and agriculture [1,2]. In agriculture, malic acid is used to solubilize aluminum phosphate in soil [4]. Annually, the global market ranges from 40,000–60,000 metric tons of malic acid with the growth rate for malic acid increasing by 4% annually [5]. The value of the market has been estimated as $130 million [5]. It has been reported that the retail price of a pound of malic acid ranges from $1.80 to $2.00 [3]. The current industrial method of malic acid synthesis is petroleum-based. The commercial production of malic acid involves the hydration of maleic anhydride (from the oxidation of benzene or butane) under higher pressure and temperature which synthesizes a racemic mix of D- and L-malic acid where the isomers require chiral resolution [1,2].

An alternate method of synthesizing malic acid is by utilizing the enzyme fumarate hydratase or *Saccharomyces cerevisiae* cells containing the overexpressed gene for fumarate hydratase to catalyze the conversion of fumarate to malate [2,3,6]. The most recent method of synthesizing malic acid is by the acid hydrolysis of poly(β-L-malic acid) (PMA) that is metabolically synthesized by microbes such as *Aureobasidium pullulans* [2]. PMA can also be synthesized from malic acid or it can be hydrolyzed to malic acid. PMA may be useful in the production of biodegradable polymers for use in pharmaceuticals and agriculture since it is a water-soluble biopolymer [7–10]. Also, low molecular weight PMA has been reported to be a protease inhibitor [11]. Green chemistry approaches to produce malic acid from low value biomass or processing coproducts have begun being investigated. Such low value products could help to reduce the price of malic acid compared to its production using petroleum-based products. By lowering its price/pound, biobased malic acid production could more effectively compete with petroleum-based production for use in various food or non-food products. This review of biobased malic acid production first explores its metabolic synthesis in various microbes. Next, possible production from biofuel-related coproducts, lignocellulosic biomass or polymalic acid is examined.

2. Pathways of Malic Acid Biosynthesis

Possible pathways of malic acid biosynthesis have been explored and it has been concluded that there are likely three pathways by which microorganisms synthesize malic acid (Figure 1). The first pathway is characterized as a reductive pathway (Figure 1). This pathway is thought to involve pyruvate carboxylase and malate dehydrogenase where pyruvate initially undergoes carboxylation to oxaloacetate and oxaloacetate is reduced to malate [2]. This pathway is thought to require carbon dioxide derived from the addition of a carbonate salt to the culture medium or sparging the medium with CO_2. Therefore, this pathway requires fixation of CO_2 but is neutral relative to ATP. There is evidence that this pathway is occurring in eukaryotes such *Aspergillus flavus*, *Aspergillus oryzae*, *Candida glabrata*, *Penicillium* spp. and *Saccharomyces cerevisiae* and in the prokaryotes *Bacillus subtilis* and a metabolically engineered strain of *Escherichia coli* [2,12–14]. In *S. cerevisiae*, it was shown that pyruvate carboxylase and malate dehydrogenase activities were elevated in a strain exhibiting elevated malic acid production [13]. In a strain of the thermophilic soil bacterium, *Thermobifida fusca* muC, phosphoenolpyruvate is converted to oxaloacetate by phosphoenolpyruvate carboxylase and the oxaloacetate reduced to malate by malate dehydrogenase [5]. Thus far, the microorganisms utilizing the reductive pathway produced the highest malic acid levels and yields [2,12–14]. The second pathway of malate biosynthesis by microbes involves the glyoxylate pathway where two molecules of acetyl-CoA are used (Figure 1). The glyoxylate pathway enzyme isocitrate lyase converts isocitrate into succinic acid and glyoxylate. The second enzyme of the pathway malate synthetase catalyzes a reaction involving acetyl-CoA, glyoxylate and water to form malate and Co-A. Carboxylation of malate is necessary for oxaloacetate production to occur and the glyoxylate cycle to continue. It has been shown that *S. cerevisiae* utilizes this pathway to produce malate [2,15]. As shown in Figure 1, the oxidative pathway of malic acid synthesis by microbes involves the tricarboxylic acid cycle [2]. Acetyl-CoA undergoes condensation with oxaloacetate and enters the tricarboxylic acid cycle as citrate until malate is formed with the release of two CO_2 [2]. It is thought that *S. cerevisiae* can use this pathway to produce excess malate particularly if fumarase is overexpressed in the yeast cells [2,6]. Of the three pathways to synthesize malic acid, the reductive pathway appears to be the primary pathway utilized by most organisms.

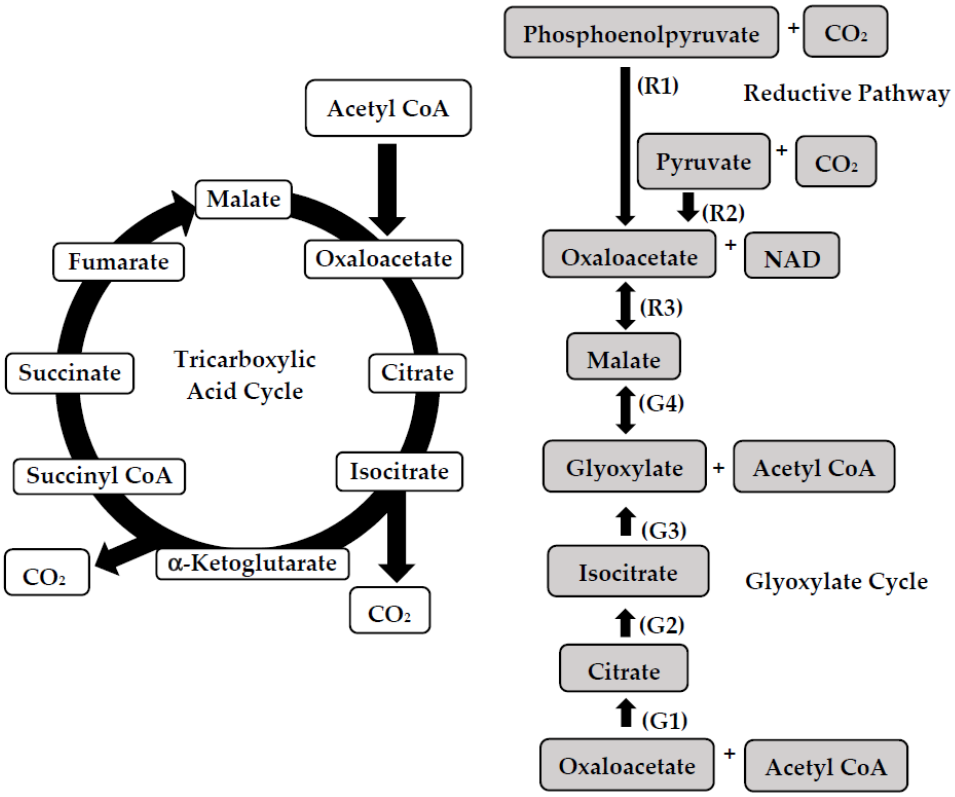

Figure 1. Three metabolic pathways in microorganisms utilized to synthesize malic acid. The tricarboxylic acid cycle which forms malate oxidatively is indicated on the right. The reductive pathway (R) of malic acid synthesis involves phosphoenolpyruvate carboxykinase (R1), pyruvate carboxylase (R2) and malate dehydrogenase (R3). The glyoxylate cycle (G) pathway enzymes include citrate synthase (G1), aconitase (G2), isocitrate lyase (G3) and malate synthase (G4). The tricarboxylic acid pathway is mitochondrial (no shading) while the reductive and glyoxylate cycle pathways are cytosolic (shaded in gray).

3. Microbial Malic Acid Production from Sugars

The microbial production of malic acid from sugars has been investigated and it has been concluded that glucose is the optimum fermentable sugar in the presence of a carbonate salt when the nitrogen concentration is limiting in the medium [16–18]. The carbonate salt serves a dual function as a neutralizing agent and a source of CO_2 [16]. Fermentable sugars have been shown to support malic acid production by species of the fungus *Aspergillus* [16–18]. Prior studies demonstrated malic acid production by *A. flavus* ATCC 13697. This strain grown in a 10 L fermentor (0.5 volume of air/volume of culture/min with a stirring speed of 300 rpm) for 192 h at 25 °C from 10% (*w*/*v*) glucose as the carbon source and 80 g/L calcium carbonate produced 36.4 g/L malic acid [16]. Malic acid was synthesized from pyruvate with pyruvate carboxylase being involved [17,19]. In a 16 L fermentor, ATCC 13697 produced 113 g/L malic acid using 12% (*w*/*v*) glucose as a carbon source and 60 g/L calcium carbonate after 190 h (0.5 volume of air/volume of culture/min with a stirring speed of 400 rpm producing a 20% dissolved oxygen concentration) at 32 °C [18]. The problem associated with large-scale malic acid production by *A. flavus* is that the fungus can produce aflatoxins and it would not be granted a "generally regarded as safe status" [2]. It was determined that nitrogen-limiting conditions the *A. oryzae* strains stimulated malic acid production and that peptone as a nitrogen source increased malic acid production from glucose [20]. When the incubation temperature for *A. oryzae* DSM 1863 growth was increased from 30 to 35 °C, its malic acid production in a 2 L fermentor (0.5 volume of air/volume of culture/min with a stirring speed of 300 rpm) after 168 h on 114 g/L (*w*/*v*) glucose and 90 g/L (*w*/*v*)

calcium carbonate was increased by 1.7-fold [21]. The five carbon sugar xylose also supported malic acid production by *A. oryzae* DSM 1863 but its molar yield (0.56) was lower than on glucose (0.82) as a carbon source [21]. In the fungus *Rhizopus delemar*, production of 60 g/L malic acid from corn straw hydrolysate (100 g/L glucose and 25 g/L xylose) occurred in 60 h at 30 °C in a 3 L fermentor (aeration provided by magnetic stirring) [22]. For the fungus *Penicillium sclerotium* K301, a level of 88.6 or 92 g/L calcium malate was produced from 142 g/L (*w*/*v*) glucose as a carbon source and 50 g/L (*w*/*v*) calcium carbonate in shake flasks or in a 10 L fermentor (aeration rate of 8 L/min with stirring speed of 300 rpm) after 96 or 72 h at 28 °C, respectively [23]. Another species of the fungus, namely *Penicillium viticola* 152 (isolated from a marine environment) was shown to produce 168 g/L calcium malate in a medium containing 140 g/L (*w*/*v*) glucose, 40 g/L (*w*/*v*) calcium carbonate and 0.5% (*v*/*v*) corn steep liquor in a 10 L fermentor (aeration rate of 8 L/min with stirring speed of 300 rpm) for 96 h at 28 °C [4]. Metabolic engineering of various species is also being explored as a way to produce high levels of malic acid under controlled conditions in a fermentor [2,5,12,14,15,24,25]. It was reported that a metabolically engineered *A. oryzae* ATCC 56747 produced approximately four-fold higher levels of malic acid than did the wild type strain [12]. In the genetically engineered strain, pyruvate carboxylase (Figure 1), malate dehydrogenase (Figure 1) and the C4-dicarboxylate transporters were overexpressed [12]. The metabolically engineered ATCC 56747 produced 154 g/L malic acid after 164 h at 34 °C (1 volume of air/volume of culture/min with an agitation rate set at 500 rpm) when the dissolved oxygen level was maintained above 50% [12]. Genetic manipulation of strains of *S. cerevisiae* to increase yield (0.42 mol malic acid/mol glucose) and *E. coli* (1.42 mol malic acid/mol glucose) have also been used to improve their malic acid production [14,15]. Although the metabolically engineered *S. cerevisiae* strain was grown on 188 g/L glucose at 30 °C in the presence of 500 mM calcium carbonate at pH 6.0, the genetically modified *E. coli* strain produced a higher yield on only 50 g/L glucose at 37 °C in the presence of 100 mM potassium bicarbonate at pH 7.0 [14,15]. Similarly, the metabolic engineering of *C. glabrata* CCTC M202019 to overproduce the activities of pyruvate carboxylase, malate dehydrogenase and a malate transporter caused a several fold increase in malate production compared to its parent strain [25]. In a metabolically engineered strain of *A. oryzae*, malic acid overproduction was observed when pyruvate carboxylase and malate dehydrogenase were increased by several-fold compared to its parent strain [24].

4. Malic acid Production from Biofuel-Related Coproducts

It has been shown that biofuel-related coproducts can be used to support microbial malic acid production (Table 1) but the level of malic acid produced will depend on the type of coproduct and the ability of the microbe to utilize or tolerate it. One coproduct that can be used to support microbial malic acid production is thin stillage which is formed during the dry milling of corn to produce ethanol (Table 1). Thin stillage is the coproduct that is recovered from whole stillage after removal of yeast cells by centrifugation and its composition includes a high percentage of glycerol and about 1% nitrogen [26]. It is usually mixed with wet distillers' grains and dried to produce dried distillers' grains with solubles [26]. To improve the economics of ethanol production, thin stillage may not be mixed with wet distillers' grains and dried [26]. Instead, wet distillers' grains is marketed alone which has reduced the value of thin stillage as a coproduct. This stillage does contain glycerol which is suitable for microbial fermentation [27]. A prior study has examined the utilization of thin stillage to support malic acid production by species of *Aspergillus* [28]. A number of strains were tested for their ability to synthesize malic acid from thin stillage including *Aspergillus niger* ATCC 9029, ATCC 9142, ATCC 10577 and *A. flavus* ATCC 13697 [28]. Of the strains screened, the yields of malic acid from thin stillage by *A. niger* ATCC 9142 and ATCC 10577 were highest (0.79 g malic acid/g glucose and glycerol in thin stillage) [28]. It was concluded that the ethanol production coproduct thin stillage could be utilized for fungal malic acid production [28].

Crude glycerol is another coproduct that could be utilized for the synthesis of malic acid and is produced during the production of biodiesel (Table 1). The production of biodiesel involves the esterification of a vegetable oil using methanol at an alkaline pH using heat [29]. This process results in the coproduct crude glycerol being formed [30]. The composition of crude glycerol includes glycerol, fatty acids, and methyl esters of fatty acids [29]. Approximately 10% of the coproduct formed during the esterification process is characterized as the crude glycerol fraction. It is expected that substantial volumes of crude glycerol will have to be processed as waste with the current global annual production of biodiesel being more than 30 million tons [31]. With crude glycerol being valued at a current price of $0.05/pound [32], there will be large volumes of crude glycerol available as low-cost feedstock for microbial fermentation. Crude glycerol has been reported to be a substrate for microbial production of malic acid [33]. It has been shown that crude glycerol derived from the production of biodiesel was capable of supporting fungal malic acid synthesis [1,2,30]. In a recent study, *A. niger* ATCC 9142, ATCC 10577 and ATCC 12846 were found to be capable of producing malic acid on 10% (*v*/*v*) crude glycerol after 192 h at 25 °C [34]. Of the three strains, *A. niger* ATCC 12846 produced the highest malic acid concentration from crude glycerol [34]. The ability to produce malic acid from crude glycerol did not seem to be highly correlated with cellular biomass since ATCC 10577 produced a higher biomass level than did ATCC 12846 after 192 h at 25 °C [34]. Although *A. oryzae* DSM 1863 was also capable of producing 45 g/L malic acid from 84 g/L (*w*/*v*) glycerol after 353 h at 35 °C, its ability to utilize crude glycerol as a substrate for malic acid production has not been investigated [19]. The fungus *Ustilago trichophora* TZ1 was investigated for its ability to produce malic acid from glycerol and crude glycerol (Table 1) in recent studies [35,36]. It was noted that high concentrations of glycerol or crude glycerol had an inhibitory effect on the fungal growth rate. The medium was supplemented with calcium carbonate to provide carbon dioxide as well as to maintain the pH above 5.4 to allow fungal malic acid production to occur [35]. As the level of crude glycerol in the culture was increased from 100 to 200 g/L (*w*/*v*), malic acid production by *U. trichophora* was diminished [36]. It was concluded that for the fungus to effectively grow on crude glycerol that it will likely require adaptive evolution of strain TZ1.

Table 1. Malic acid production by microorganisms grown on biofuel-processing coproducts or hydrolyzed lignocellulosic biomass.

Coproduct/Hydrolyzed Lignocellulosic Biomass	Microorganism	Growth Conditions	Malic Acid (g/L)	Yield (g/g)	Reference
Corn stover	*T. fusca* muC-16	55 °C	21.5	0.43	[4]
Corn straw	*R. delemar* HF-119	30 °C	60.0	0.48	[22]
	R. delemar HF-121	30 °C	121.8	0.97	[22]
Thin stillage	*A. flavus* ATCC 13697	25 °C	10.2	0.48	[28]
	A. niger ATCC 9029	25 °C	1.0	0.05	[28]
	A. niger ATCC 9142	25 °C	16.9	0.79	[28]
	A. niger ATCC 10577	25 °C	16.4	0.79	[28]
Crude glycerol	*A. niger* ATCC 9142	25 °C	16.5	0.17[1]	[34]
	A. niger ATCC 10577	25 °C	20.3	0.20[1]	[34]
	A. niger ATCC 12846	25 °C	23.5	0.24[1]	[34]
	U. trichophora TZ1	30 °C	108.0	0.26	[35]
Syngas (plant biomass)	*C. ljungdahli* DSM 13528/ *A. oryzae* DSM 1863	25 °C	1.1	0.17	[37]
Biooil (plant biomass)	*A. oryzae* DSM 1863	32 °C	0.0	0.0	[38]

[1] The malic acid yields produced by *A. niger* ATCC 9142, ATCC 10577 and ATCC 12846 on 10% (*v*/*v*) crude glycerol represent unpublished data.

5. Lignocellulosic Biomass-Based Malic Acid Production

An alternative to using coproducts from biofuel production could be the utilization of plant biomass hydrolysates or biooils to synthesize malic acid (Table 1). The challenge of using lignocellulosic biomass instead of a processing coproduct to microbially produce malic acid is the cost of enzymes (cellulases and xylanases) to degrade the biomass to fermentable sugars (glucose or xylose). The hydrolysis of lignocellulosic biomass by physical, chemical and enzymatic treatments to produce glucose from cellulose and xylose from xylan represents a green chemistry approach since plant biomass is biorenewable [39–41]. Hydrolyzed corn straw has been used as a substrate for the fungus *R. delemar* strain HF-119 to synthesize malic acid (Table 1). The corn straw was heat at 160 °C for 20 s and treated with 0.4% sulfuric acid [22]. The resultant solid residue was treated with cellulase and β-glucosidase at 120 rpm for 72 h at 52 °C to produce glucose and xylose [22]. A fluoroacetate mutant strain HF-121 was isolated (Table 1) and shown to be capable of producing 120.5 g/L malic acid from the corn straw hydrolysate (125 g/L mixed sugars) at 30 °C within 60 h [22]. This strain was noted to utilize glucose and xylose as carbon sources more efficiently than strain HF-119 [22]. Xylose could also be utilized by other fungi such as *A. oryzae* DSM 1863 to synthesize malic acid [21]. Syngas can be converted to malic acid using a sequential approach [37]. Initially, a medium sparged with a syngas produced from the gasification of straw is used to anaerobically grow *Clostridium ljungdahli* at 37 °C to synthesize acetate. The acetate is aerobically converted by *A. oryzae* to 4.34 g/L malic acid in the absence of nitrogen (Table 1). It was also shown that bioreactor production of malic acid was possible using this two-step process [37]. A pyrolysis oil produced from the fast hydrolysis of wheat straw has been shown to support malic acid production by *A. oryzae* DSM 1863 (Table 1) depending on the growth conditions [38]. It was noted that the strain could tolerate 1%–2% biooil but the biooil alone did not support fungal malic acid synthesis [38]. The addition of pyrolysis oil (0.5%) to a glucose-containing medium supported malic aid production by the strain but inclusion of higher biooil concentrations (1%–2%) decreased malic acid levels in the same medium after 7 days at 32 °C [38]. Inhibitors within the biooil were found to be responsible for the diminution in malic acid production by the strain [38]. It has been shown that a strain of the filamentous soil bacterium *T. fusca* muC could produce malic acid from cellulose at 55 °C. The bacterium produces a cellulase allowing it to degrade cellulose over a wide pH range [5]. The presence of yeast extract in the medium stimulated cell growth and malate production at 55 °C at 250 rpm [5]. It was also determined that the histidine phosphocarrier protein was the repressor of malic acid production in *T. fusca* muC [42]. A metabolic engineered strain *T. fusca* muC-16 that contained the pyruvate carboxylase gene from *Corynebacterium glutamicum* ATCC 13032 produced a malic acid yield that was 48% higher than its parent strain [5]. The metabolically engineered strain could ferment cellulose and corn stover into malic acid [5]. The strain muC-16 produced about 63 g/L malic acid from 100 g/L cellulose after 124 h at 55 °C. This strain could also convert corn stover (26.9% glucan and 19.3% xylan) to 21.5 g/L malic acid after 120 h at 55 °C (Table 1). It is clear that malic acid can be synthesized by microorganisms from lignocellulosic biomass but additional research on the utilization of other types of hydrolyzed biomass, such as wood, for microbial malic production is necessary.

6. Malic Acid Derived from poly(β-L-malic acid) (PMA) Production

The fungal production of PMA for eventual conversion to malic acid may be the most promising and economical method to synthesize malic acid. It has been shown that malic acid can also be produced from the acid hydrolysis of PMA synthesized by *A. pullulans* [7–9]. PMA is hydrolyzed at 85 °C to pure malic acid using 2 M sulfuric acid [8]. When calcium carbonate was used as a neutralizing agent during the fungal production of PMA, the molecular weight of the PMA was increased compared to using sodium carbonate [11]. It was demonstrated that alkaline peroxide-treated corn straw or wheat straw supported PMA by four strains of *A. pullulans* [7]. If 3% (*w*/*v*) calcium carbonate and hydrolytic enzymes (such as cellulase, xylanase and glucosidase) were added to medium, PMA production by the strains was enhanced within 7 days at 25 °C [7]. *A. pullulans* NRRL 50383 produced greater

than 20 g/L PMA within 7 days at 25 °C under optimal conditions [7]. The mutant strain ZX-10, isolated from *A. pullulans* NRRL Y-2311-1, produced PMA (76.2 g/L) and malic acid (87.6 g/L) from 180 g/L glucose after 140 h at 25 °C in a stirred-tank 5 L bioreactor [8]. The yield of malic acid from glucose was 0.49 g malic acid/g glucose [7]. Another strain of *A. pullulans*, namely YJ6-11, produced 28.6 g/L PMA or 32.4 g/L malic acid from a corncob hydrolysate (produced by treatment with 1% sulfuric acid and treatment with cellulase and xylanase) after 72 h at 25 °C in a 5 L fermentor [43]. The corncob hydrolysate contained 90 g/L mixed sugars including glucose, xylose and arabinose [43]. The advantage of using strain YJ6-11 is that it is able to utilize glucose or xylose at a comparable rate to produce PMA [43]. Using an aerobic fibrous bed bioreactor, this strain was further adapted to corncob hydrolysate by fermentation on the hydrolysate (96 g/L glucose, 54% xylose, 2.5% furfurals and 1.8% acetic acid) for 864 h with a new isolate (CCTCC M2012223) of the original strain being isolated [10]. The strain CCTCC M2012223 was isolated by adaption to increasing concentrations of corncob hydrolysate that contained the growth inhibitors furfural, 5-hydroxymethyfurfural, formic acid and acetic acid [10]. The resultant evolved strain was more resistant to growth inhibition by the known inhibitors [10]. In another study, PMA production by strain CCTCC M2012223 was found to be influenced by nitrogen availability [44]. PMA production was maximum at 36 g/L when the isolated strain was grown on the hydrolysate (110 g/L sugar mix) and ammonium sulfate (2 g/L) in the fibrous bed reactor at 25 °C with aeration for 120 h [44]. Free cells (stirred tank bioreactor) or immobilized cells (in an aerobic fibrous bed bioreactor) of *A. pullulans* CCTCC M2012223 could produce PMA in a medium containing raw sweet potato hydrolysate (120 g/L carbohydrate), yeast extract and citrate [44]. Using free cells of the strain, 29.6 g/L PMA was produced after 120 h at 25 °C [45]. In the immobilized cell system, the strain produced 57.5 g/L PMA after 156 h at 25 °C [45]. PMA can also be produced by *A. pullulans* ZX-10 on sugarcane molasses (44% sucrose, 6% glucose and 5% fructose) [46]. The strain produced 52.6 g/L PMA after 187.5 h at 25 °C during batch fermentation [46]. In a 5 L fermentor, the strain produced 116.3 g/L PMA after 340 h at 25 °C [46]. Under fed-batch conditions, *A. pullulans* ZX-10 was also capable of utilizing a soybean hull hydrolysate (26.8 g/L carbohydrate) supplemented with corn steep liquor (10 g/L, *w*/*v*) at 25 °C for 168 h to produce 31.3 g/L malic acid from 27.2 g/L PMA [47]. In the same study, *A. pullulans* ZX-10 was capable of growing on soybean molasses (26.8 g/L carbohydrate) under fed-batch conditions for about 260 h to produce 71.9 g/L malic acid from 62.6 g/L PMA [47]. Microbial production of PMA from agricultural biomass would seem to be as effective as direct microbial malic acid fermentation to synthesize this industrially-valuable organic acid.

7. Conclusions

In summary, an opportunity to use low value biomass or processing coproducts such as thin stillage, crude glycerol or straw, exists to synthesize the platform chemical malic acid. From a "green chemistry" perspective, it could help reduce the reliance on petroleum-based chemicals to synthesize malic acid by substituting biobased processes. The most pressing issues that have to be addressed for the biobased production of malic acid include strain development and process development. Both factors are critical for the cost of biobased malic acid to be competitive with petroleum-based malic acid. New environmentally-friendly, microbial-based approaches can only be achieved with microbes capable of elevated malic acid production in the presence of growth inhibitors in plant biomass hydrolysates or processing coproducts. Using these microbes, process development will be essential for the efficient bioconversion of biomass hydrolysates or coproducts into malic acid to effectively compete with the current petroleum-based production methods from an economic standpoint.

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References

1. Mondala, A.H. Direct fungal fermentation of lignocellulosic biomass into itaconic, fumaric and malic acids: Current and future prospects. *J. Ind. Microbiol. Biotechnol.* **2015**, *42*, 487–506. [CrossRef] [PubMed]
2. Chi, Z.; Wang, Z.-P.; Wang, G.-Y.; Khan, I.; Chi, Z.-M. Microbial biosynthesis and secretion of L-malic acid and its applications. *Crit. Rev. Biotechnol.* **2016**, *36*, 99–107. [CrossRef] [PubMed]
3. West, T.P. Microbial malic acid production: Exploring new avenues of synthesizing a commercially-valuable chemical. *J. Microb. Biochem. Technol.* **2016**, *8*, 321. [CrossRef]
4. Khan, I.; Nazir, K.; Wang, Z.-P.; Liu, G.-L.; Chi, Z.-M. Calcium malate overproduction by *Penicillium viticola* 152 using the medium containing corn steep liquor. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 1539–1546. [CrossRef] [PubMed]
5. Deng, Y.; Mao, Y.; Zhang, X. Metabolic engineering of a laboratory-evolved *Thermobifida fusca* muC strain for malic acid production on cellulose and minimal treated lignocellulose. *Biotechnol. Prog.* **2016**, *32*, 14–20. [CrossRef] [PubMed]
6. Neufeld, R.J.; Peleg, Y.; Rokem, J.S.; Pines, O.; Goldberg, I. L-Malic acid formation by immobilized *Saccharomyces cerevisiae* amplified for fumarase. *Enzym. Microb. Technol.* **1991**, *13*, 991–996. [CrossRef]
7. Leathers, T.D.; Manitchotpisit, P. Production of poly(β-L-malic acid) (PMA) from agricultural biomass substrates by *Aureobasidium pullulans*. *Biotechnol. Lett.* **2013**, *35*, 83–89. [CrossRef] [PubMed]
8. Zou, X.; Zhou, Y.; Yang, S.T. Production of polymalic acid and malic acid by *Aureobasidium pullulans*. *Biotechnol. Bioeng.* **2013**, *110*, 2105–2113. [CrossRef] [PubMed]
9. Chi, Z.; Liu, G.L.; Liu, C.-G.; Chi, Z.-M. Poly(β-L-malic acid) (PMA) from *Aureobasidium* spp. and its current proceedings. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 3841–3851. [CrossRef] [PubMed]
10. Zou, X.; Wang, Y.; Tu, G.; Zan, Z.; Wu, X. Adaption and transcriptome analysis of *Aureobasidium pullulans* in corncob hydrolysate for increased inhibitor tolerance to malic acid production. *PLoS ONE* **2015**. [CrossRef]
11. Cao, W.; Chen, X.; Luo, J.; Yin, J.; Qiao, C.; Wan, Y. High molecular weight poly(β-L-malic acid) produced by *A. pullulans* with Ca^{2+} added repeated batch culture. *Int. J. Biol. Macromol.* **2016**, *85*, 192–199. [CrossRef] [PubMed]
12. Brown, S.H.; Bashkirova, L.; Berka, R.; Chandler, T.; Doty, T.; McCall, K.; McCulloch, M.; McFarland, S.; Thompson, S.; Yaver, D.; Berry, A. Metabolic engineering of *Aspergillus oryzae* NRRL 3488 for increased production of L-malic acid. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 8903–8912. [CrossRef] [PubMed]
13. Oba, T.; Kusomoto, K.; Kichise, Y.; Izumoto, E.; Nakayama, S.; Tashiro, K.; Kuhara, S.; Kitagaki, H. Variations in mitochondrial membrane potential correlate with malic acid production by natural isolates of *Saccharomyces cerevisiae* sake strains. *FEMS Yeast Res.* **2014**, *14*, 789–796. [CrossRef] [PubMed]
14. Zhang, X.; Wang, X.; Shanmugam, K.T.; Ingram, L.O. L-Malate production by metabolically engineered *Escherichia coli*. *Appl. Environ. Microbiol.* **2011**, *77*, 427–434. [CrossRef] [PubMed]
15. Zelle, R.M.; Hulster, E.; van Winden, W.A.; de Waard, P.; Dijkema, C.; Winkler, A.A.; Geertman, J.M.A.; van Dijken, J.P.; Pronk, J.T.; van Maris, A.J. Malic acid production by *Saccharomyces cerevisiae*: Engineering of pyruvate carboxylation, oxaloacetate reduction, and malate export. *Appl. Environ. Microbiol.* **2008**, *74*, 2766–2777. [CrossRef] [PubMed]
16. Peleg, Y.; Stieglitz, B.; Goldberg, I. Malic acid accumulation by *Aspergillus flavus*. I. Biochemical aspects of acid biosynthesis. *Appl. Microbiol. Biotechnol.* **1988**, *28*, 69–75.
17. Peleg, Y.; Barak, A.; Scrutton, M.C.; Goldberg, I. Malic acid accumulation by *Aspergillus flavus*. III. ^{13}C NMR and isoenzymes analyses. *Appl. Microbiol. Biotechnol.* **1989**, *30*, 176–183. [CrossRef]
18. Battat, E.; Peleg, Y.; Bercowitz, A.; Rokem, J.S.; Goldberg, I. Optimization of L-malic acid production by *Aspergillus flavus* in a stirred fermentor. *Biotechnol. Bioeng.* **1991**, *37*, 1108–1116. [CrossRef] [PubMed]
19. Bercovitz, A.; Peleg, Y; Battat, E.; Rokem, J.S.; Goldberg, I. Localization of pyruvate carboxylase in organic acid producing *Aspergillus* strains. *Appl. Environ. Microbiol.* **1990**, *56*, 1594–1597. [PubMed]
20. Knuf, C.; Nookaew, I.; Brown, S.; McCulloch, M.; Berry, A.; Nielsen, J. Investigation of malic acid production in *Aspergillus oryzae* under nitrogen starvation conditions. *Appl. Environ. Microbiol.* **2013**, *79*, 6050–6058. [CrossRef] [PubMed]
21. Ochsenreither, K.; Fischer, C.; Neumann, A.; Syldatk, C. Process characterization and influence of alternative carbon source and carbon-to-nitrogen ratio on organic acid production by *Aspergillus oryzae* DSM1863. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 5449–5460. [CrossRef] [PubMed]

22. Li, X.; Liu, Y.; Yang, Y.; Zhang, H.; Wang, H.; Wu, Y.; Zhang, M.; Sun, T.; Cheng, J.; Wu, X.; Pan, L.; Jiang, S.; Wu, H. High levels of malic acid production by the conversion of corn straw hydrolyte using an isolated Rhizopus Delemar strain. *Biotechnol. Bioprocess Eng.* **2014**, *19*, 478–492. [CrossRef]
23. Wang, Z.-P.; Wang, G.-Y.; Khan, I.; Chi, Z.-M. High-level production of calcium malate from glucose by *Penicillium sclerotium* K302. *Bioresour. Technol.* **2013**, *143*, 674–677. [CrossRef] [PubMed]
24. Knuf, C.; Nookaew, I.; Remmers, I.; Khoomrung, S.; Brown, S.; Berry, A.; Nielsen, J. Physiological characterization of the high malic acid-producing *Aspergillus oryzae* strain 21031-68. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 3517–3527. [CrossRef] [PubMed]
25. Chen, X.; Xu, G.; Xu, N.; Zou, W.; Zhu, P.; Liu, Liming; Chen, J. Metabolic engineering of *Torulopsis glabrata* for malate production. *Metab. Eng.* **2013**, *19*, 10–16. [CrossRef] [PubMed]
26. Kim, Y.; Mosier, N.S.; Hendrickson, R.; Ezeji, T.; Blaschek, H.; Dien, B.; Cotta, M.; Dale, B.; Ladisch, M.R. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. *Bioresour. Technol.* **2008**, *99*, 5165–5176. [CrossRef] [PubMed]
27. Gonzalez, R.; Campbell, P.; Wong, M. Production of ethanol from thin stillage by metabolically engineered *Escherichia coli*. *Biotechnol. Lett.* **2010**, *32*, 405–411. [CrossRef] [PubMed]
28. West, T.P. Malic acid production from thin stillage by *Aspergillus* species. *Biotechnol. Lett.* **2011**, *33*, 2463–2467. [CrossRef] [PubMed]
29. Tapasvi, D.; Wiesenborn, D.; Gustafson, C. Process model for biodiesel production from various feedstocks. *Trans. ASAE* **2005**, *48*, 2215–2221. [CrossRef]
30. West, T.P. Crude glycerol: A feedstock for organic acid production by microbial bioconversion. *J. Microb. Biochem. Technol.* **2012**. [CrossRef]
31. Quispe, C.A.G; Coronado, C.J.R.; Carvalho, J.A., Jr. Glycerol: Production, consumption, prices, characterization and new trends in combustion. *Renew. Sustain. Energy Rev.* **2013**, *27*, 475–493. [CrossRef]
32. Yang, F.; Hanna, M.A.; Sun, R. Value-added uses for crude glycerol—A byproduct of biodiesel production. *Biotechnol. Biofuels* **2012**, *5*, 13–22. [CrossRef] [PubMed]
33. Dobson, R.; Gray, V.; Rumbold, K. Microbial utilization of crude glycerol for the production of value-added products. *J. Ind. Microbiol. Biotechnol.* **2012**, *39*, 217–226. [CrossRef] [PubMed]
34. West, T.P. Fungal biotransformation of crude glycerol into malic acid. *Z. Naturforsch. C* **2015**, *70*, 165–167. [CrossRef] [PubMed]
35. Zambanini, T.; Kleineberg, W.; Sarikaya, E.; Buescher, J.M.; Meurer, G.; Wierckx, N.; Blank, L.B. Enhanced malic acid production from glycerol with high-density *Ustilago trichophora* TZ1 cultivations. *Biotechnol. Biofuels* **2016**, *9*, 135. [CrossRef] [PubMed]
36. Zambanini, T.; Sarikaya, E.; Kleineberg, W.; Buescher, J.M.; Meurer, G.; Wierckx, N.; Blank, L.B. Efficient malic acid production from glycerol with *Ustilago trichophora* TZ1. *Biotechnol. Biofuels* **2016**, *9*, 67. [CrossRef] [PubMed]
37. Oswald, F.; Dorsam, S.; Veith, N.; Zwick, M.; Neumann, A.; Ochsenreither, K.; Syldatk, C. Sequential mixed cultures: From syngas to malic acid. *Front. Microbiol.* **2016**. [CrossRef] [PubMed]
38. Dorsam, S.; Kirchhoff, J.; Bigalke, M.; Dahmen, N.; Syldatk, C.; Ochsenreither, K. Evaluation of pyrolysis oil as carbon source for fungal fermentation. *Front. Microbiol.* **2016**. [CrossRef] [PubMed]
39. Zhang, Y.H.P. Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 367–375. [CrossRef] [PubMed]
40. West, T.P. Xylitol production by *Candida* species grown on a grass hydrolysate. *World J. Microbiol. Biotechnol.* **2009**, *25*, 913–916. [CrossRef]
41. West, T.P. Effect of nitrogen source concentration on curdlan production by *Agrobacterium* sp. ATCC 31749 grown on prairie cordgrass hydrolysates. *Prep. Biochem. Biotechnol.* **2016**, *46*, 85–90. [CrossRef] [PubMed]
42. Deng, Y.; Lin, J.; Mao, Y.; Zhang, X. Systematic analysis of an evolved *Thermobifida fusca muC* producing malic acid on organic and inorganic nitrogen sources. *Sci. Rep.* **2016**, *6*, 30025. [CrossRef] [PubMed]
43. Zou, X.; Yang, J.; Tian, X.; Guo, M.; Li, Z.; Li, Y. Production of polymalic acid and malic acid from xylose and corncob hydrolysate by a novel *Aureobasidium pullulans* YJ 6-11 strain. *Process Biochem.* **2016**, *51*, 16–23. [CrossRef]
44. Wang, Y.; Song, X.; Zhang, Y.; Wang, B.; Zou, X. Effects of nitrogen availability on polymalic acid biosynthesis in the yeast-like fungus *Aureobasidium pullulans*. *Microb. Cell Fact.* **2016**, *15*, 146. [CrossRef] [PubMed]

45. Zan, Z.; Zou, X. Efficient production of polymalic acid from raw sweet potato hydrolysate with immobilized cells of *Aureobasidium pullulans* CCTC M2012223 in aerobic fibrous bed bioreactor. *J. Chem. Technol. Biotechnol.* **2013**, *88*, 1822–1827. [CrossRef]
46. Wei, P.; Cheng, C.; Lin, M.; Zhou, Y.; Yang, S.-T. Production of poly(malic acid) from sugarcane juice in fermentation by *Aureobasidium pullulans*: Kinetics and process economics. *Bioresour. Technol.* **2017**, *224*, 581–589. [CrossRef] [PubMed]
47. Cheng, C.; Zhou, Y.; Lin, M.; Wei, P.; Yang, S.-T. Polymalic acid fermentation from *Aureobasidium pullulans* for malic acid production from soybean hull and soy molasses: Fermentation kinetics and economic analysis. *Bioresour. Technol.* **2017**, *223*, 166–174. [CrossRef] [PubMed]