



Biotechnological production of itaconic acid—things you have to know

Anja Kuenz¹ · Susan Krull¹

Received: 26 January 2018 / Revised: 23 February 2018 / Accepted: 26 February 2018 / Published online: 13 March 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Itaconic acid is one of the basic chemicals for the polymer industry, which can be produced on the basis of renewable raw materials. Since the middle of the twentieth century, itaconic acid has been produced industrially using the filamentous fungus *Aspergillus terreus*. But the demand for the organic acid is low due to the high production costs compared to alternative petrochemical manufactured raw materials. The high production costs are based on a low final titer, low productivities, and the usage of pure sugars, purified molasses, or starch hydrolysates, since the fungus reacts very sensitively to impurities in a culture medium. This review provides a comprehensive overview of the most recent developments, including a spectrum of studied microorganisms and their capabilities for the production of itaconic acid. The technological achievements in the biotechnological production of itaconic acid are presented. Particular attention is paid to current achievements in terms of suitable alternative substrates and their applicability in fermentation processes. Also, the pathway of itaconic acid and especially the influences on the fermentation process, which must be known in order to achieve a high final titer of itaconic acid, a yield close to the theoretical yield, and high productivity.

Keywords Itaconic acid · Wild-type strains · Genetically engineered strains · Fermentation strategies · Renewable resources

Introduction

In 1837, itaconic acid was discovered as a thermal decomposition product of citric acid (Baup 1837). Itaconic acid is a single unsaturated dicarbonic acid and also known as methylene succinic acid (Fig. 1). Due to its chemical structure, one unsaturated double bond and two carboxyl groups, itaconic acid can be converted to a number of high-value bio-based chemicals or materials generated from carbohydrates (Werpy et al. 2004). The threefold functional structure allows a variety of reactions and applications and it is able to replace petrochemical-based methacrylic or acrylic acid (Magalhães et al. 2016b; Robert and Friebe 2016). Because of the two carboxyl groups, itaconic acid has two pK_a values, $pK_{a1} = 3.84$ and $pK_{a2} = 5.55$ at 25 °C. In an aqueous solution, the concentration of each dissociated form depends on the present pH value. Mainly the non-dissociated acid occurs at pH values lower than pH 2, wherein at a pH value higher than pH 7, the doubly dissociated itaconate is for the most part present.

Between pH 2 and pH 7, a mixture of all dissociation forms occurs (Krull et al. 2017b; Rychtera and Wase 1981). Itaconic acid crystallizes in rhombic double pyramids and has the physical constants given in Table 1 (Willke and Vorlop 2001).

The varieties of functional groups in the itaconic acid molecule make them an effective intermediate in the preparation of complex organic compounds. Different reactions, like salt formation with metals, esterification with alcohols, anhydride formation, addition reactions, and polymerization, are possible.

The industrially versatile usability of itaconic acid and its derivatives, like 3-methylpyrrolidin, 2-methyl-1,4-butanediamine, itaconic acid diamide, 3-methyltetrahydrofuran, or unsaturated esters, are reflected in the wide range of applications, e.g., styrene-butadiene rubber, synthetic latex, super-absorbent polymers, unsaturated polyester resins, and detergents. The field of application of these products is widespread and ranges from the paint, lacquer, and paper industries through to hygiene and medical products up to the construction sector. Usage in coatings, plasticizers, chemical fibers, or other plastics is also known (Delidovich et al. 2016; Klement and Büchs 2013; Kumar et al. 2017; Magalhães et al. 2016b; Okabe et al. 2009; Robert and Friebe 2016; Saha 2017; Willke and Vorlop 2001).

In the case of a further reduction of the itaconic acid production costs, a future application of itaconic acid could be

✉ Anja Kuenz
anja.kuenz@thuenen.de

¹ Thünen - Institute of Agricultural Technology, Bundesallee 47, 38116 Braunschweig, Germany

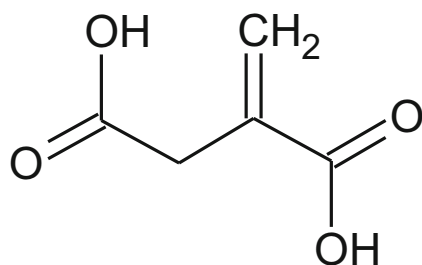


Fig. 1 Structure of itaconic acid

methyl methacrylate production (thermoplastics). Thus, the estimated projected market of itaconic acid in 2020 could reach nearly 407,000 t/a, with a market value of approximately US\$500 million (Weastra sro 2013). If the itaconic acid were to be produced on the basis of alternative raw materials, the production costs of polyitaconic acid would have to decline from US\$3/kg to US\$1.50/kg to replace petrochemical-based polyacrylic acid (Durant 2009).

Chemical production of itaconic acid

A chemical method to produce itaconic acid is the pyrolysis of citric acid and the hydrolysis of these anhydrides (Baup 1837). Itaconic acid was also produced using the decarboxylation of aconitic acid and the name itaconic acid was obtained as an anagram. Other chemical syntheses, such as distillation of citric acid and further treatment of the yielded anhydrides, oxidation of mesityl oxides with the subsequent isomerization of the formed citraconic acid, or oxidation of isoprene, are known (Willke and Vorlop 2001).

Biotechnological production of itaconic acid

The first biotechnological production of itaconic acid was described by Kinoshita (1932) with *Aspergillus itaconicus*. Calam (1939) achieved higher itaconic acid concentrations with *Aspergillus terreus* as a surface culture. Charles Pfizer Co. applied the first patent in 1945 for an industrial production process of itaconic acid by a submerged cultivation of *A. terreus* (Kane et al. 1945) and built the first production plant in Brooklyn, NY, USA, in 1955.

Table 1 Properties of itaconic acid

Chemical formula	C ₅ H ₆ O ₄
Molar mass	130.1 g/mol
Appearance	White and crystalline
Density	1.63 g/cm ³
Melting point	162–167 °C
Heating point	268 °C
pK _a values	3.84 and 5.55
Solubility in H ₂ O (20 °C)	83 g/L

Further production facilities were located in Japan, England, and France. Due to the demands for the lowest possible production costs, the production facilities were increasingly relocated to the Asia-Pacific region (Okabe et al. 2009; Weastra sro 2013).

As a result of continuous optimization of the itaconic acid production with *A. terreus* since the 1960s, the biotechnological production process is more economical compared to the chemical processes, and the chemical processes cannot compete with the biotechnological process (Tate 1981).

The worldwide estimated itaconic acid production in 2011 was 41,400 t with a market value of US\$74.5 million (Weastra sro 2013). The price per kilogram of itaconic acid varies between US\$1.80 and US\$2.00 depending on the manufacturer and quality of the product.

It was assumed that a phosphate limitation is required for itaconic acid overproduction by *A. terreus* (Klement and Büchs 2013; Rychtera and Wase 1981; Welter 2000). However, this thesis has been refuted. Neither a phosphate nor a nitrogen limitation is necessary to initiate a production of itaconic acid (Hevekerl et al. 2014b; Krull et al. 2017b). The trigger for the overproduction of itaconic acid in *A. terreus* is still unknown. However, the biosynthesis including the enzymes and transporters has been very well studied and described.

For the formation of itaconic acid (Fig. 2), sugars, such as glucose, xylose, or arabinose, are uptakes from *A. terreus* and converted to pyruvate in the cytosol via the metabolic pathway glycolysis and the pentose phosphate pathway (Eimhjellen and Larsen 1955; Saha et al. 2017). The pyruvate is transported into the mitochondrion and catalyzed over the citrate cycle to citrate and cis-aconitate. Via a citrate malate antiporter or mitochondrial tricarboxylate transporter, cis-aconitate gets into the cytosol, where it is converted to itaconate by cis-aconitate decarboxylase (CAD) (Bonnarme et al. 1995; Jaklitsch et al. 1991; Steiger et al. 2013). Afterwards, the itaconate enters the medium using a transporter, which presumably belongs to the group of major facilitator superfamily proteins (Li et al. 2011). Thus, in the reaction of 1 mol glucose, xylose, or arabinose, 1 mol of itaconic acid can be formed. This corresponds to a maximum theoretical yield of 72% (Saha et al. 2017).

In addition to the industrially used *A. terreus* strain, other organisms, like *Ustilaginaceae*, are also able to form itaconic acid (Geiser et al. 2014; Guevarra and Tabuchi 1990; Haskins et al. 1955). According to the current literature, the trigger of itaconic acid overproduction in *Ustilago maydis* is ammonium limitation (Maassen et al. 2014). The metabolic pathway of *U. maydis* to form itaconic acid is comparable to the metabolic pathway of *A. terreus*. However, according to Geiser et al. (2016a) *U. maydis* converts cis-aconitate in the cytosol first to trans-aconitate using a cytosolic aconitate- Δ -isomerase (Adi1). Trans-aconitate is then catalyzed by a novel enzyme,

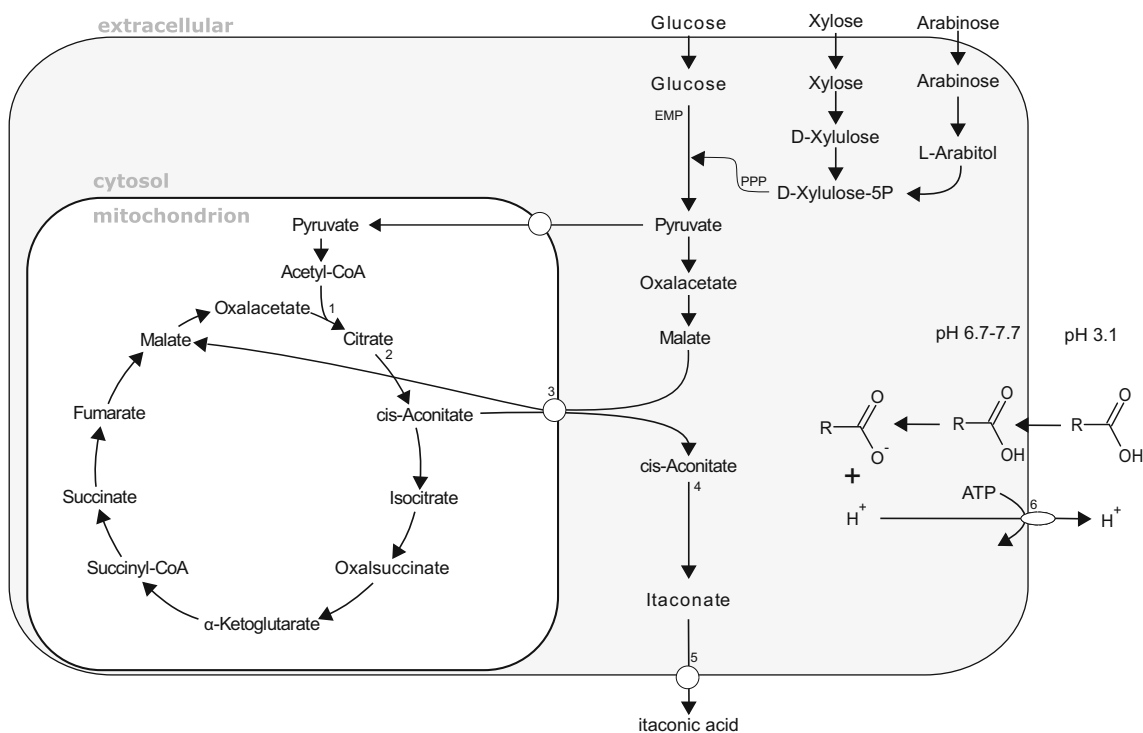


Fig. 2 Simplified illustration of the pathway of itaconic acid in *A. terreus* (Bonname et al. 1995; Jaklitsch et al. 1991; Li et al. 2011) and equilibrium of weak acids in the medium and cytoplasm (Lambert and Stratford 1999). (1) Citrate synthase. (2) Isocitrate dehydrogenase. (3) Citrate

malate antiporter, mitochondrial tricarboxylate transporter (mtt). (4) Cis-aconitate decarboxylase (CAD). (5) Major facilitator superfamily protein (mfs). (6) H^+ -ATPase. EMP Embden-Meyerhof-Parnas, PPP pentose phosphate pathway

trans-aconitate decarboxylase (Tad1), into itaconic acid. Geiser et al. (2016a) showed that *U. maydis* synthesized itaconate via the unusual intermediate trans-aconitate. In addition to the genes for the enzymes aconitate- Δ - isomerase and trans-aconitate decarboxylase, also a mitochondrial TCA transporter (mtt1) and an itaconate transporter protein (Itp1), which belong to the major Facilitator superfamily, were found in the same gene cluster of itaconic acid synthesis (Geiser et al. 2016a). Furthermore, Zambanini et al. (2017a) examined two of the native promoters, P_{Tad1} and P_{mtt1} from the itaconate cluster of *U. maydis*. It is known that the trigger of itaconic acid overproduction in *U. maydis* is ammonium limitation, and it is postulated that these two promoters are well suited to induce gene expression in response to the nitrogen limitation that is coupled to the itaconic acid production phase. This investigation provides a new set of genetic tools leading to further improvement of the organic acid production using metabolic engineering strategies with *Ustilago* (Zambanini et al. 2017a).

Wild-type strains

In addition to the industrially used *A. terreus* strain, other organisms, like *Ustilaginaceae*, are able to form itaconic acid (Geiser et al. 2014; Guevarra and Tabuchi 1990; Haskins et al. 1955). Also known as itaconic acid producers are yeasts of the

species *Pseudozyma* (Levinson et al. 2006; Specht et al. 2014), *Candida* (Tabuchi et al. 1981), and the fungi *Rhodotorula* (Kawamura et al. 1981) and *Helicobasidium* (Sayama et al. 1994). From these alternative itaconic acid producers, Specht et al. (2014) achieved the highest itaconic acid concentration of 75 g/L with an oscillating dissolved oxygen concentration. The highest currently published titer of itaconic acid produced with the genus *Ustilago* is 44.5 g/L (Maassen et al. 2014). Both titers are well below achieved concentration of 130–160 g/L itaconic acid with *A. terreus* (Hevekerl et al. 2014b; Karaffa et al. 2015; Krull et al. 2017b). There are also mammalian macrophages (Strelko et al. 2011) that are known to produce itaconic acid in traces. An overview of various itaconic acid-producing wild-type strains is given in Table 2.

Genetically engineered strains

In addition to the natural itaconic acid-producing strains, genetically modified organisms are increasingly produced. Plasmids with the full itaconic acid biosynthesis cluster are introduced into microorganisms that possess the citrate cycle, to overexpress the production of itaconic acid. That includes enzymes, like the key enzyme cis-aconitate decarboxylase (CAD), which catalyze cis-aconitate to itaconic acid (Kanamasa et al. 2008; Li et al. 2011). Also important

Table 2 Itaconic acid production of wild-type strains and strains generated by random mutagenesis

Microorganism	Substrate	Itaconic acid [g/L]	Yield [g/g] ^b	Productivity [g/L/h]	Reference
<i>Aspergillus terreus</i> DSM 23081	Glucose	129	0.58	1.15	(Hevekerl et al. 2014b)
<i>Aspergillus terreus</i> NRRL 1960	Glucose	130	–	–	(Karaffa et al. 2015)
<i>Aspergillus terreus</i> DSM 23081	Glucose	160	0.46	1	(Krull et al. 2017b)
<i>A. terreus</i> SKR10 N45 ^a	Hydrolyzed corn starch	50	0.42	0.35	(Reddy and Singh 2002)
<i>Aspergillus terreus</i> R104 ^a	Glucose	52.7	0.72	0.55	(Shin et al. 2013)
<i>Aspergillus terreus</i> IFO-6365 TN-484 ^d	Glucose	82.3	0.54	0.57	(Yahiro et al. 1995)
<i>Candida</i> sp. strain B-1 ^a	Glucose	35	–	0.29	(Tabuchi et al. 1981)
<i>Helicobasidium</i> sp.	–	–	–	–	(Sayama et al. 1994)
<i>Pseudozyma antarctica</i> NRRL Y-7808	Glucose	30	0.38	–	(Levinson et al. 2006)
<i>Pseudozyma tsukubaensis</i> H488	Glucose	74.7	0.49	0.36	(Specht et al. 2014)
<i>Ustilago cynodontis</i> K470	Glucose	28.4	–	–	(Guevara and Tabuchi 1990)
<i>Ustilago maydis</i> DSM 17144	Glucose	44.5	0.24	0.31	(Maassen et al. 2014)
<i>Ustilago rabenhorstina</i> IFO 8995	Glucose	15.7	–	–	(Guevara and Tabuchi 1990)

^a Strains generated by random mutagenesis

^b Itaconate per consumed glucose

transporter systems, including the transport of cis-aconitate from the mitochondrion into the cytosol (*mttA*) and the transport of itaconic acid out of the cell (*mfsA*), are transferred to organisms which are not naturally itaconic acid producers (Li et al. 2011). An overview of various itaconic acid-producing genetically modified organisms is given in Table 3. The itaconic acid production by genetically engineered microorganisms is generally based on the following major strategies:

- Using itaconic acid-producing wild-type strains:

Aspergillus niger, the natural citric acid producer (typical industrial citric acid fermentation up to 200 g/L (Roehr et al. 1996)), is phylogenetically a close relative of *A. terreus*, but without the key enzyme cis-aconitate decarboxylase and therefore not able to produce itaconic acid. An increased

production of citric acid was yielded with a modified *pfkA* gene in relation to the parental strain. Tevz et al. inserted the modified *A. niger pfkA* genes into the *A. terreus* ATCC 20542 strain and increased the average productivity between 48 and 96 h and the final titer of itaconic acid after 168 h to 0.44 g/L/h and 45.5 g/L compared to the parental strain with 0.079 g/L/h and 21.4 g/L itaconic acid (Tevz et al. 2010). According to the literature, the strain *A. terreus* ATCC 20542 is a wild-type parent strain used for industrial lovastatin-overproducing strains (Alberts et al. 1980; Lai et al. 2007; Monaghan et al. 1981). To study the biosynthesis mechanism and increase the itaconic acid production, Huang et al. provided a gene-targeting system as an efficient platform of sequential and multiple genetic modifications in *A. terreus* (Huang et al. 2016).

The natural itaconic acid-producing wild-type strain *U. maydis* also produces 2-hydroxyparaconate (Geiser et al.

Table 3 Itaconic acid production of genetically modified organisms (GMOs)

Microorganism	Substrate	Itaconic acid [g/L]	Yield [g/g]	Productivity [g/L/h]	Reference
<i>Aspergillus niger</i> AB1.13 CitB#99	Glucose	26.2	0.37 ^b	–	(Hossain et al. 2016)
<i>Aspergillus terreus</i> A729	Glucose	45.5	–	–	(Tevz et al. 2010)
<i>Corynebacterium glutamicum</i> AO-2/ <i>pEKEEx2-malEcadopt</i>	Glucose	7.8	0.29	0.16	(Otten et al. 2015)
<i>Escherichia coli</i> ita23	Glucose	32	0.49 ^b	0.27	(Harder et al. 2016)
<i>Saccharomyces cerevisiae</i>	Glucose	0.168	–	–	(Blazeck et al. 2014)
<i>Synechocystis</i> sp. PCC6803	CO ₂	0.0145	–	–	(Chin et al. 2015)
<i>U. maydis</i> MB215 Δ <i>cyp3 P_{etef}ria1</i>	Glucose	63.2	0.23 ^b	0.38	(Geiser et al. 2016b)
<i>Yarrowia lipolytica</i>	Glucose	4.6	0.058	0.045 ^a	(Blazeck et al. 2015)

^a Maximum productivity

^b Itaconate per consumed glucose

2016b). Geiser et al. proposed that the conversion of itaconic acid to 2-hydroxyparaconate is catalyzed by the P450 monooxygenase Cyp3, encoded by *cyp3*. Due to the deletion of *cyp3* and the simultaneous overexpression of the gene cluster regulator *ria1*, an itaconate hyper producer strain (4.5-fold more itaconate, without the side product 2-hydroxyparaconate) was generated. The combination with a process optimization, led to an increased final itaconate titer, productivity, and yield (Geiser et al. 2016b).

Zambanini et al. (2017b) shifts the organic acid production towards itaconate using metabolic engineering. The transcriptional regulator and mitochondrial transporter, *ria1* or *mtt1*, from the itaconate cluster of *U. maydis* were overexpressed in *Ustilago vetiveriae*, which resulted in 2.0-fold (*ria1*) and 1.5-fold (*mtt1*) higher itaconic acid titer compared to the wild-type strain. Additionally, the malate production was reduced by 75 and 41%, respectively (Zambanini et al. 2017b).

- Using non-itaconic acid-producing wild-type strains:

According to the literature, several approaches are published that use metabolic engineering and *A. niger* as a host to increase the itaconic acid production (Blumhoff et al. 2013; Hossain et al. 2016; Li et al. 2012; van der Straat et al. 2013; van der Straat et al. 2014). So far, the highest achieved titer of itaconic acid with a genetically engineered *A. niger* is 26.2 g/L (Hossain et al. 2016). Hossein et al. overexpressed a putative cytosolic citrate synthase *citB* in *A. niger* strains which contain the full itaconic acid biosynthesis cluster. Using that approach, they achieved a maximum productivity of 0.35 g/L/h without the formation of any side products (Hossain et al. 2016).

Corynebacterium glutamicum is widely used as a host for the industrial production of L-glutamate and L-lysine. It is used as a model organism in industrial biotechnology, but not able to produce itaconic acid because of the absence of the key enzyme cis-aconitate decarboxylase (CAD). Expression of the CAD gene from *A. terreus* followed by optimizing the CAD activity as well as cultivation conditions, and reduction of the isocitrate dehydrogenase activity, yielding a final titer of 7.8 g/L itaconic acid and a maximum volumetric productivity of 0.27 g/L/h with a genetically engineered *C. glutamicum* (Otten et al. 2015).

To improve the itaconic acid production, the approach of using faster-growing organisms was conducted. Therefore, genetical engineering of *Escherichia coli*, as a well-examined and fast-growing organism, was carried out. The generated strain *E. coli* ita23 (MG1655 $\Delta aceA$ $\Delta sucCD$ $\Delta pykA$ $\Delta pykF$ Δpta $\Delta Picd::cam$ P2 pCadCs; plasmid pCadCs: BBa_J23100_ *cadA* *gltA*_T, KanR, ColE1) produced 32 g/L itaconic acid after 120 h and a maximum yield of 0.77 mol/mol_{glucose} (0.56 g/g_{glucose}). This is the highest titer reported so far using heterologous itaconic acid production (Harder et al. 2016).

Blazeck et al. increased the itaconic acid production in *S. cerevisiae* using a computationally guided genetic manipulation in order to increase the metabolic capacity. Three genetic knockouts ($\Delta ade3$ $\Delta bna2$ $\Delta tes1$), combined with a higher cell density fermentation, improved the itaconic acid final titer to 168 mg/L (Blazeck et al. 2014).

Chin et al. used CO₂ as the carbon source for the photo-synthetic production of itaconic acid with the engineered cyanobacterium *Synechocystis* sp. PCC6803 strain, expressing the key enzyme cis-aconitate decarboxylase (pJAK12-cad).

Due to the natural ability to accumulate citric acid intermediates as well as the tolerance against lower pH values, *Yarrowia lipolytica* was engineered to produce itaconic acid. Blazeck et al. expressed the key enzyme cis-aconitate decarboxylase as well as cytosolic aconitase enzymes and generated the PO1f leucine⁺uracil⁺ CAD (2 \times) ACO_{no}MLS Epi CAD Epi strain that yielded 4.6 g/L itaconic acid (Blazeck et al. 2015).

Influences on the fermentation process

An economical itaconic acid production requires a high final titer of itaconic acid, a yield close to the theoretical yield, and a high productivity. To achieve these goals, it is essential to know the parameters that influence these factors and to carry out the cultivation under reproducible conditions. In the following section, the most important factors influencing the biotechnological production of itaconic acid are described in more detail.

Morphology

The morphology of filamentous fungi influences the productivity in submersed culture. The reproduction of the filamentous fungus *Aspergillus terreus* is asexual, either through the formation of conidium spores or via chlamydo spores (Deak et al. 2009). The formation of conidium spores is described in surface and submerged cultures (Fuchs and Schlegel 2006; Hevekerl 2016; Hevekerl et al. 2014b).

Chlamydo spores are formed in submerged cultures. A pH shift during a cultivation of *A. terreus* led to a massive formation of submerged conidia. These conidia were derived from the formation of chlamydo spores, and also from the formation of submerged conidiophores. The formation of the conidiophores was observed when NaOH and KOH were used as lye. The addition of an equivalent amount of sodium ions to the fermentation broth at the time of the pH shift in the form of NaCl did not result in the formation of any conidium. The use of ammonia solution as lye for the pH shift resulted in the formation of chlamydo spores, but no conidiophores were visible (Hevekerl 2016; Hevekerl et al. 2014b). The chlamydo spores are twice as large at 4–7 μ m as the conidial spores at 2–4 μ m (Deak et al. 2009; Hevekerl 2016).

The spores of *A. terreus* form germ tubes, which become hyphae. The subsequent growth of the hyphae takes place in the form of hyphae growth at the hypha-apexes or about the division of the hypha-apexes (the top of the hyphae). A network-like structure, the mycelium, is formed through the growth and fusion of hyphae (Kück et al. 2009).

Due to chemical and physical influences, *A. terreus* grows in different morphological forms in submerged cultures. The morphology ranges from branched mycelium filaments (filamentous growth) to densely interwoven mycelium masses (pellets) (Papagianni 2004). According to the literature, loose pellets with a diameter of 0.1–0.5 mm show the highest productivity and yield of itaconic acid (Gyamerah 1995a). The morphology of more compact or larger pellets leads to limitations regarding oxygen supply and the supply with the nutrients. Additionally, the diffusion of the metabolic products will be a problem. Especially a limitation of oxygen supply should be avoided in the production of itaconic acid (Gyamerah 1995a; Kuenz et al. 2012). Moreover, the formation of mycelium causes limitations due to increased viscosity that results in a poorer mixing of the fermentation broth.

In addition to the industrially used *A. terreus* strain, other organisms, like *Ustilaginaceae*, are able to form itaconic acid. The best-described species is *U. maydis*. It serves as a model organism for the study of pathogenesis in plants (Bölker 2001) and was fully sequenced in 2006 (Kamper et al. 2006). The growth of *U. maydis* is dimorphic. The non-pathogenic haploid cells can reproduce by budding, and show a yeast-like appearance or form pseudohyphae or true hyphae (Begerow et al. 2006; Bölker 2001). Using *U. maydis* for the production of itaconic acid, a yeast-like unicellular growth has been described throughout the literature (Guevarra and Tabuchi 1990; Klement and Büchs 2013; Maassen et al. 2014).

Medium components

Important for the productivity of the most frequently used commercial itaconic acid producer *A. terreus* is the shaped morphology which is immensely influenced by several medium components. Iron, zinc, calcium, cobalt, nickel, and manganese ions in the medium can have an especially strong influence on the morphology of *A. terreus* (Batti and Schweiger 1963; Gyamerah 1995a; Karaffa et al. 2015; Kobayashi 1978; Kuenz et al. 2012; Lockwood and Reeves 1945). For example, too low calcium concentrations lead to very compact pellets, whereas a concentration of 20 g/L $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ yield an almost pseudo-, yeast-like morphology (Kuenz et al. 2012). With regard to manganese ions, Karaffa et al. (2015) recommend a medium with a concentration of lower than 3 $\mu\text{g/L}$. Higher manganese concentrations lead to a changed morphology in the form of a loose, highly branched mycelium, resulting in reduced itaconic acid yield and productivity. Thus, the influence of manganese and other

heavy metal ions on the morphology, and thus on the production, of itaconic acid with *A. terreus* is very similar to the production of citric acid with *A. niger* (Choudhary and Pirt 1966; Clark et al. 1966; Tomlinson et al. 1950). In addition to the morphology, heavy metals also inhibit relevant enzymes, such as the key enzyme CAD, in itaconic acid production (Dwiarti et al. 2002).

pH value

In the literature, very different approaches regarding the pH value and process strategies are described using *A. terreus* for the cultivation of itaconic acid. For the initial pH, a broad range is used from pH 2 to pH 5.9 (Gyamerah 1995b; Park et al. 1993; Rychtera and Wase 1981; Yahiro et al. 1995). The pH has an influence on the germination speed of the conidia. pH values lower than pH 3 slow the germination and thereby postpone the itaconic acid production (Batti and Schweiger 1963; Hevekerl et al. 2014b). After the germination of the spores, the ammonium ions, which serve as the nitrogen source, are consumed. This process releases protons, which cause a drop of the pH in the unbuffered media (Mattey 1992). Low pH values during the growth of the mycelium are necessary to acquire the ability to produce itaconic acid. Independent of the pH of the production medium, the mycelium grown at pH 2 was able to produce itaconic acid, whereas the mycelium grown at pH 6 did not produce itaconic acid (Larsen and Eimhjellen 1955). Larsen and Eimhjellen suggested that under these low pH values, relevant enzyme systems are activated which are necessary for the itaconic acid production. After the start of itaconic acid production, different pH-related strategies lead to a similar final titer of about 90 g/L itaconic acid. Kuenz et al. (2012) adjusted the pH value to pH 3.1 at the beginning of the cultivation and did not subsequently adjust the pH, so that it dropped below pH 2. On the other hand, Batti and Schweiger (1963) once raised the pH value to pH 3.2 with ammonia water after 5.8 days.

A significant increase in the final titer of itaconic acid, far beyond 90 g/L, was achieved by changing the pH value during the production phase of the cultivation. A rise in the pH value, due to a single pH shift or a pH control, increased the final itaconic acid titer from 87 to 146 g/L (Hevekerl et al. 2014b). Currently, the highest yield of itaconic acid of 160 g/L is achieved under a pH control in the production phase to pH 3.4 (Krull et al. 2017b). The degree of the dissociation of itaconic acid shifts with increasing pH from H_2IA to HIA^- and finally to IA^{2-} depending on the pH value. Krull et al. showed a correlation between the increased final titer of itaconic acid with the increased itaconic acid form of HIA^- , which resulted from the pH control to 3.4 in the production phase. So far, a further increase of the pH value in order to produce more HIA^- and thus higher final itaconic acid titer was not successful because of a changed morphology (Krull et al. 2017b).

Using *Ustilaginaceae* for the production of itaconic acid, the pH dependency of the itaconic acid is described in the literature (Geiser et al. 2014). The well-described *U. maydis* yielded a titer of 44.5 g/L itaconic acid under pH-controlled cultivations at pH 6 (Maassen et al. 2014).

Oxygen supply

The formation of itaconic acid is strongly dependent on a sufficient oxygen supply. For the itaconic acid production with *A. terreus*, it is known that a short interruption of the aeration results in the decrease of the productivity or even to a complete stop of the itaconic acid production (Gyamerah 1995b; Kuenz et al. 2012; Larsen and Eimhjellen 1955; Nelson et al. 1952; Pfeifer et al. 1952). The critical duration until a negative effect occurs can be as short as 3 min, but can differ, depending on the used cultivation parameters. According to literature, centrifugation or filtration of the mycelium leads to the loss of itaconic acid production (Larsen and Eimhjellen 1955). Kuenz et al. 2012 showed that independent of the used itaconic acid-producing strain, the sufficient and especially continuous supply of oxygen during the production phase is essential. The fungus is able to regain the ability to produce itaconic acid after an oxygen limitation. After 20–24 h, the itaconic acid production starts again, but with a much lower productivity (Gyamerah 1995b; Pfeifer et al. 1952). This delay is presumably caused by the synthesis of new protein systems in the cells (Gyamerah 1995b).

During the production phase, the dissolved oxygen level only slightly affected the itaconic acid production with *A. terreus*. Park et al. (1993) varied the dissolved oxygen level between 20 and 60% and found no influence on the reached itaconic acid concentrations, but a slightly better yield at lower dissolved oxygen levels (Park et al. 1993).

Other itaconic acid-producing organisms have not yet been studied for the influence of oxygen levels and limitations. However, it can be assumed that for the cultivation of *U. maydis*, a high input of oxygen is necessary, due to the high shaking frequencies used in different studies (Carstensen et al. 2013; Geiser et al. 2016b; Guevarra and Tabuchi 1990; Maassen et al. 2014).

Side products

The production of itaconic acid using the fungus *A. terreus* depends, among other things, on the pH value. During itaconic acid production at pH 2.1, malic acid occurred as a side product, whereas during the cultivation at pH 6, no itaconic acid was formed but malic acid, succinic acid, and fumaric acid were accumulated (Larsen and Eimhjellen 1955). Changing the pH value during the production phase of the cultivation significantly influences the titer of itaconic acid as well as the production of side products. With a start pH of

3.1 and no control during the cultivation, the share of the side products are about 3.3%, consisting of 97% malic acid and α -ketoglutaric acid as the major side products, as well as *cis*- and *trans*-aconitate. Carrying out a pH shift after 2.1 days, the share of the side products decreased (Hevekerl et al. 2014b).

Using *U. maydis* as an itaconic acid-producing strain, the trigger of the overproduction of itaconic acid is ammonium limitation (Maassen et al. 2014). However, nitrogen limitation also causes the formation of intracellular and extracellular lipids (Haskins et al. 1955; Hewald et al. 2005; Maassen et al. 2014). Especially the formation of extracellular glycolipids, such as ustilagic acid in form of oil drops or mannosylerythritol lipid (MEL) in the form of needle-like crystals, has been demonstrated (Bölker et al. 2008).

Guevarra and Tabuchi 1990 provide an overview of *Ustilaginaceae*, including the product formation. In addition to the main product itaconic acid, *U. maydis* formed itatartaric acid, 1-2-hydroxyparaconic acid, and malic acid.

U. cynodontis and *U. rabenhorstiana* produced, in addition to itaconic acid, itatartaric acid, 1-2-hydroxyparaconic acid, and erythritol. *U. spermophora* formed the side products malic acid and succinic acid (Guevarra and Tabuchi 1990). Geiser et al. 2014 screened 68 *Ustilaginaceae* regarding their production of organic acids, polyols, and glycolipids. They identified *U. maydis* and *U. vetiveriae* as producers of itaconate, malate, and succinate as well as *U. cynodontis* and *U. xerochloae* as producers of itaconate, malate, succinate, and erythritol (Geiser et al. 2014).

Fermentation of alternative substrates

A. terreus is able to utilize not only different substrates like glucose, xylose, sucrose but also glycerol or ethanol for growth and itaconic acid production (Eimhjellen and Larsen 1955). The highest yields are achieved with sucrose and glucose (Hevekerl et al. 2014b; Karaffa et al. 2015; Krull et al. 2017b).

From 100 *A. terreus* strains, 20 strains showed good itaconic acid production from xylose and arabinose (Saha et al. 2017). Further on, 20 *A. terreus* strains were evaluated regarding their ability to convert mannose and galactose to itaconic acid. One of these strains, *A. terreus* NRRL 1971 showed, with 36.4 g/L itaconic acid and a yield of 0.46 g/g_{mannose}, the highest itaconic acid titer reported so far using mannose as the carbon source (Saha and Kennedy 2017a).

To optimize the itaconic acid production, the incubation system was scaled down in microtiter plates (Hevekerl et al. 2014a). To examine the ability of *A. terreus* to utilize different monosaccharides, like glucose, xylose, arabinose, galactose, and rhamnose, a combination of these monosaccharides, the influence of enzyme formulation, as well as the influence of sugar degradation products, microtiter plates were used successfully (Krull et al. 2017a; Saha and Kennedy 2017b).

As an alternative to the pure sugars, sugar beet and sugarcane molasses (Batti and Schweiger 1963; Kane et al. 1945), as well as starch hydrolysates (Cros and Schneider 1993; Dwiarti et al. 2007; Petruccioli et al. 1999; Reddy and Singh 2002; Yahiro et al. 1997), were successfully used for itaconic acid production. In addition, Reddy and Singh (2002) successfully produced itaconic acid on sugar extract from fruit peel waste.

Cultivation on starch hydrolysates or sugar extracts is significantly more successful because no sugar degradation products can be produced as an inhibitor by harsh pretreatment conditions of the lignocellulosic biomass. On the other hand, these harsh conditions are necessary for lignocellulose in order to break up the significantly more complex structure of lignocellulose compared to starch (Jönsson et al. 2013). The resulting inhibiting sugar degradation products or other impurities must be removed by usually complex purification processes in order to achieve a sufficient production of itaconic acid with *A. terreus*. Another possibility is to render the strain more resistant to potential inhibitors by mutagenesis. However, it is very difficult to compare different microorganisms cultured on different lignocellulose hydrolysates with different purification methods (Palmqvist and Hahn-Hägerdal 2000).

Li et al. (2016) used an undetoxified enzymatic hydrolysate of steam-exploded corn stover as the sole carbon source. In addition to glucose and xylose, this also contained formic acid and acetic acid. With an *A. terreus* wild-type strain, only 0.54 g/L itaconic acid is formed, presumably due to the inhibiting effect of the acids. By mutagenesis of the strain through atmospheric and room temperature plasma, the titer increased to 19.3 g/L itaconic acid (Li et al. 2016). By using activated carbon, Wu et al. (2017) reduced the concentration of furfural of the used wheat bran hydrolysate and increased the production of itaconic acid from 8 to 34.2 g/L. A further increase to 49.6 g/L itaconic acid was achieved using an *A. terreus* mutant (Wu et al. 2017). Krull et al. (2017a) succeeded the cultivation with a wild-type strain of *A. terreus* and without the use of activated carbon or ion exchanger and achieved 23.3 g/L itaconic acid with a yield of 0.27 (w/w) using a wheat chaff hydrolysate. Due to a deft hydrolysis method, the formation of inhibitors was avoided, and as a result of this, a complex purification was not necessary. Both process variables, final titer, and yield, could additionally increase to 27.7 g/L itaconic acid and 0.41 (w/w) by an optional removal of cations (Krull et al. 2017a). Pedrosa et al. (2017) used a phosphoric hydrolysate of rice husks and produced 1.9 g/L itaconic acid with a yield of 49 mg/g_{sugar} using *A. terreus* ATCC 10020. Jimenez-Quero et al. (2016) carried out different pretreatments of two lignocellulosic biomasses, wheat bran and corn cobs. Depending on the method, different concentrations of sugars, metals, or inhibitors, and different itaconic acid yields were obtained, depending on the *Aspergillus* strains they tested.

Another attempt is the utilization of the carbon source glycerol for the itaconic acid production. Compared to glucose as a substrate, Kuenz (2008) obtained with glycerol a similar growth of *A. terreus* DSM 23081 and 69.7 g/L itaconic acid after 15 days of cultivation. Juy et al. (2010) also used glycerol as the sole carbon source and *A. terreus* MJL05, isolated from vendee's lands. After varying and optimizing C:N, N:P, and C:P ratios the itaconic acid production was improved to 27.6 g/L itaconic acid with a productivity of 0.192 g/L/h and a yield of 0.44 g/g_{glycerol}. Zambanini et al. (2017b) achieved an itaconic titer of 34.7 g/L combined with 46.2 g/L malate from 196 g/L glycerol with a productivity of 0.09 g/L/h using the strain *Ustilago vetiveriae* TZ1. Also the itaconic acid production with glycerol, using *E. coli* scvCadA_No8, has been shown to be successful. Under optimized cultivation conditions, 7.2 g/L itaconic were produced after approximately 85 h (Jeon et al. 2016). A genetically engineered *Yarrowia lipolytica* produced 2.65 g/L itaconic acid after 168 h in a nitrogen-limited medium containing 100 g/L glycerol (Wang et al. 2012).

Kim et al. (2017) described a whole-cell bioconversion of citrate to itaconate using *E. coli*, with enhanced aconitase and cis-aconitate decarboxylase activities by controlling the expression of multiple cadA genes. For this reason, the conversion of 500 mM citrate to 319.8 mM (41.6 g/L) itaconate is carried out successfully without using buffer systems or additional cofactors.

An overview of itaconic acid-producing organisms from alternative substrates is given in Table 4.

Product recovery and purification

The quality and the environmental compatibility of product isolation and purification contribute significantly to the evaluation of a biotechnical process. The following methods in the product recovery of itaconic acid have already been described in the literature:

Solids, e.g., biomass, are separated by filtration or centrifugation continuously or at the end of batch cultivations. Subsequently, the itaconic acid can be crystallized using several evaporation and cooling processes, whereby an industrial degree of purity of the itaconic acid is achieved. If activated carbon and filtrations are used in evaporation processes, itaconic acid is obtained at a high quality. Afterwards, the residual solution is treated by liquid/liquid extraction or anion exchanger (Lockwood 1975). Using glucose or sucrose as a substrate, the precipitation of insoluble itaconic acid salts with high purity by crystallization is possible (Kobayashi 1971). To reduce the cost of product recovery and purification, technologies such as ultrafiltration for mycelial separation, bipolar electro dialysis (Kobayashi et al. 1972), reverse osmosis, or ion exchange techniques have been used (Kobayashi et al. 1973; Kobayashi et al. 1980).

Table 4 Itaconic acid production from alternative substrates

Substrate, nutrient source	Purification	Microorganism	Itaconic acid [g/L]	Yield [g/g]	Productivity [g/L/h]	System	Reference
Beech wood hydrolysate	Washing step (0.5 M NaOH, 50 °C) zeolite anion and cation exchanger	<i>A. terreus</i> NRRL 1960	7.2	0.3 ^a	0.1	SF	(Tippkötter et al. 2014)
Corn stover hydrolysate	–	<i>A. terreus</i> CICC2452	0.64	0.02 ^b	0.009	SF	(Li et al. 2016)
Corn stover hydrolysate	–	<i>A. terreus</i> Mutant AT-90	19.3	0.36 ^b	0.16	SF	(Li et al. 2016)
Corn starch hydrolysate	Neutralization (CaCO ₃) filtration	<i>A. terreus</i> Mutant N45	50	0.42 ^b	0.35	SF	(Reddy and Singh 2002)
Fruit waste extract - banana	–	<i>A. terreus</i> Mutant N45	30	0.35 ^b	0.21	SF	(Reddy and Singh 2002)
Fruit waste extract - apple	–	<i>A. terreus</i> Mutant N45	32	0.37 ^b	0.22	SF	(Reddy and Singh 2002)
Rice husks hydrolysate	Neutralization (CaO(s))	<i>A. terreus</i> ATCC 10020	1.9	0.0-49 ^b	–	SF	(Pedroso et al. 2017)
Wheat bran hydrolysate	Neutralization (Ca(OH) ₂)	<i>A. terreus</i> CICC40205	8	–	–	STR	(Wu et al. 2017)
Wheat bran hydrolysate	Neutralization (Ca(OH) ₂) activated carbon	<i>A. terreus</i> CICC40205	34.2	0.41 ^c	0.29	STR	(Wu et al. 2017)
Wheat bran hydrolysate	Neutralization (Ca(OH) ₂) activated carbon	<i>A. terreus</i> CICC40205 Mutant	49.6	0.55 ^c	0.41	STR	(Wu et al. 2017)
Wheat chaff hydrolysate	Neutralization	<i>A. terreus</i> DSM 23081	23.3	0.27 ^b	0.16	SF	(Krull et al. 2017a)
Wheat chaff hydrolysate	Neutralization cation exchanger	<i>A. terreus</i> DSM 23081	27.7	0.41 ^b	0.19	SF	(Krull et al. 2017a)
Glycerol	–	<i>A. terreus</i> DSM 23081	69.7	0.48 ^b	0.19	STR	(Kuenz 2008)
Glycerol	–	<i>A. terreus</i> MJL05	27.6	0.44 ^b	0.19	STR	(Juy et al. 2010)
Glycerol	–	<i>E. coli</i> scvCadA_No8	7.2	–	0.09	STR	(Jeon et al. 2016)
Glycerol	–	<i>U. vetiveriae</i> TZ1	34.7	0.18	0.09	SF	(Zambanini et al. 2017b)
Citrate	–	<i>E. coli</i> BL21(DE3)	41.6	64% ^d	2.19	SF	(Kim et al. 2017)

SF shake flask, STR stirred tank reactor

^a Itaconate per initial glucose and xylose concentration

^b Itaconate per consumed carbon source

^c Itaconate per consumed glucose and xylose

^d Conversion of initial citrate concentration

Relating to industrial processes, the main steps of the product recovery of itaconic acid are decoloration, clarification, evaporation, and crystallization steps. The purification processes for itaconic acid were recently reviewed by Anjum et al. (2016), López-Garzón and Straathof (2014), and Magalhães et al. (2016b). A schematic diagram of the itaconic acid production and recovery process, including evaporation, crystallization steps, and recrystallization after activated carbon treatment are given by Magalhães et al. (2016b) and Okabe et al. (2009). Magalhães et al. reviewed the current state of the art of crystallization, precipitation, liquid-liquid extraction, membrane separation, and adsorption, including the comparison of the advantages and disadvantages of the separation methods (Magalhães et al. 2016b). According to Okabe et al., a high-purity itaconic acid requires solvent extraction, ion exchange, and decolorization (Okabe et al. 2009).

In addition to the already reviewed and known product recovery and purification processes, the following new findings are published. Holzhäuser et al. (2017) reduced the number of product recovery steps as well as the need of energy-intensive separation steps by using catalytic conversion within the fermentation broth. The potential of a chemo- and electrochemical reduction of itaconic acid to methylsuccinic acid using acidic media or a crude fermentation broth was under investigation. Pure itaconic acid was converted efficiently by means of chemocatalytic hydrogenation over Ru/C or RANEY® nickel. In real fermentation broths, the presence of various salts as well as glucose prevented direct chemocatalytic valorization. However, using electrochemical hydrogenation, the conversion of pure itaconic acid in a dilute sulfuric acid environment decreased only slightly compared to a real fermentation broth. A

complete conversion and yield was achieved by simple optimizations of the reaction time and the substrate concentration (Holzhäuser et al. 2017).

Schute et al. (2016) uses the new findings in the field of selective liquid-phase adsorption, in which highly hydrophobic porous materials have been used, as new possibilities for process development and demonstrates the efficiency of selective liquid-phase adsorption for the separation and purification of itaconic acid from aqueous solutions. A wide variety of different adsorbents were under investigation, with surface polarity as well as texture properties as critical parameters for their performance. Depending on the pH value, the itaconic acid occurs as an acid (H_2IA), in a single-protonated form (HIA^-) as well as in the twice-protonated form (IA^{2-}), which has a great influence on the adsorption performance. In addition, experiments were carried out on a continuously operated fixed bed adsorber and evaluated the desorption behavior (Schute et al. 2016).

Magalhães et al. (2016a) carried out the separation of itaconic acid by adsorption from aqueous solutions using two types of commercial, strongly basic ion exchange resins: Purolite A-500P and PFA-300. Parameters like the pH value, temperature, and itaconic acid concentration were varied in order to evaluate the separation process. After batch experiments, continuous adsorption experiments were performed using a fixed-bed column. A simplified mathematical model was developed; the adsorption parameters were determined and compared with the experimental data (Magalhães et al. 2016a).

Within the framework of the project BioConSept, different process techniques, like membrane, crystallization, electrodiagnosis, extraction, and adsorption, were evaluated in detail. Regarding the recovery process of itaconic acid, a process of fermentation and product recovery using freeze crystallization were investigated and tested up to bench scale (BioConSept 2016).

Economic aspects

On an industrial scale, itaconic acid is synthesized biotechnologically using the fungus *A. terreus* and a final concentration of about 80 g/L (Kumar et al. 2017; Willke and Vorlop 2001). Itaconic acid's global production is estimated at more than 80,000 t/a, and about US\$2 kg⁻¹ (Okabe et al. 2009). Reasoned by the good efficiency of this material, it is estimated that the annual production capacity will increase by 5.5% between 2016 and 2023 (Marked Report 2015). Okabe et al. (2009) provide an overview about the supply of itaconic acid including the company, location, production start, and capacity. According to Okabe et al. 2009, the largest capacities of itaconic acid production are located at Eddyville (USA, Cargill) with 30,000 t/a, at Kyogyo (Japan, Iwata

Chemicals) with 10,000 t/a, and Melle (France, Rhodia) with 10,000 t/a (Okabe et al. 2009).

Meanwhile, the itaconic acid production in the USA (Cargill), Japan (Iwata Chemicals), and France (Rhodia) have stopped and has been shifted to China (Weastra sro 2013).

Since 2000, China is an important supplier of itaconic acid. Among others, important companies and their capacities are Qingdao Kehai Biochemical Co., Ltd., and Zhejiang Guoguang Biochemistry Co., Ltd. (10,000–15,000 t/a (Tsao et al. 2010; Weastra 2013)) as well as Qingdao Langyatai Group (15,000 t/a, Jiaonan, Shandong), Shandong Kaison Biochemical (5000 t/a, Wulian, Shandong), Shandong Zhongshun Science And Technology Development Co., Ltd. (3000 t/a, Zibo, Shandong), Enjoyrong Biochemical Co.Ltd. (5000 t/a, Wulian, Rizhao, Shandong), Leader Industry Co.,Ltd. (15,000 t/a, Zhangqiu, Jinan, Shandong), and Chengdu Lake Biology Engineering Industry (4000 t/a, Sichuan) (Listofcompanies 2017; Okabe et al. 2009).

Leaf Technologies (LESAFFRE ADVANCED FERMENTATION TECHNOLOGIES, France) and Dutch DNA (The Netherlands), a TNO spin-out, entered into a research and development collaboration to develop a new itaconic acid-producing fungal strain, in order to reduce the itaconic acid production costs and to enable a wider use as a renewable chemical (LeafDutchDNO 2015a; LeafDutchDNO 2015b; McCoy 2015).

Concluding remarks

Itaconic acid is one of the basic chemicals for the polymer industry, which can be produced on the basis of renewable raw materials. Since the middle of the twentieth century, itaconic acid has been produced industrially using the filamentous fungus *Aspergillus terreus*. Compared to alternative petrochemical manufactured raw materials, the demand for biotechnological produced itaconic acid is low due to the high production costs. The costs of the biotechnological production of itaconic acid are mainly limited by low product concentration, low productivity and low yields. Particularly in recent years, many new findings have been presented in the field of biotechnical production of itaconic acid.

On the one hand, for the well-known production with the filamentous fungus *Aspergillus terreus*, the parameters influencing the production of itaconic acid, such as media components, pH value during cultivation and morphology, have been clearly pointed out. In this way it was possible to further improve the itaconic acid production with an *A. terreus* wild-type strain and achieve a similar level of concentration as in the industrial citric acid production.

On the other hand, in the field of pathways, new insights were gained in the enzyme systems involved, but above all in the field of transporter systems. This allows a better

understanding of itaconic acid production, of naturally itaconic acid-producing strains, and their metabolic engineering. Furthermore, these findings enable also the metabolic engineering of non-itaconic acid-producing wild-type strains. It remains exciting what will arise in this area and also in the area of the usage of alternative raw materials in the future and their contribution to the price reduction of biotechnologically produced itaconic acid.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J (1980) Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc Natl Acad Sci U S A* 77(7):3957–3961
- Anjum S, Tripathi S, Singh N, Gupta KS (2016) Reactive extraction a boom for itaconic acid: a review. *Int J Recent Sci Res* 7(5):11372–11376
- Batti M, Schweiger LB (1963) Process for the production of itaconic acid. United States Patent 3 078 217
- Baup S (1837) Über eine neue Pyrogen- Citronensäure, und über Benennung der Pyrogen Säure überhaupt. *Ann Chim Phys* 19:29–38
- Begerow D, Stoll M, Bauer R (2006) A phylogenetic hypothesis of *Ustilaginomycotina* based on multiple gene analyses and morphological data. *Mycologia* 98(6):906–916
- BioConSept (2016) Final report summary—BIOCONSEPT (integration of bio-conversion and separation technology for the production and application of platform chemicals from 2nd generation biomass). Project ID: 289194; funded under: FP7-KBBE
- Blazeck J, Hill A, Jamoussi M, Pan A, Miller J, Alper HS (2015) Metabolic engineering of *Yarrowia lipolytica* for itaconic acid production. *Metab Eng* 32:66–73. <https://doi.org/10.1016/j.ymben.2015.09.005>
- Blazeck J, Miller J, Pan A, Gengler J, Holden C, Jamoussi M, Alper HS (2014) Metabolic engineering of *Saccharomyces cerevisiae* for itaconic acid production. *Appl Microbiol Biot* 98(19):8155–8164. <https://doi.org/10.1007/s00253-014-5895-0>
- Blumhoff ML, Steiger MG, Mattanovich D, Sauer M (2013) Targeting enzymes to the right compartment: metabolic engineering for itaconic acid production by *Aspergillus niger*. *Metab Eng* 19:26–32. <https://doi.org/10.1016/j.ymben.2013.05.003>
- Bölker M (2001) *Ustilago maydis*—a valuable model system for the study of fungal dimorphism and virulence. *Microbiol-Sgm* 147: 1395–1401
- Bölker M, Basse CW, Schirawski J (2008) *Ustilago maydis* secondary metabolism—from genomics to biochemistry. *Fungal Genet Biol* 45:S88–S93
- Bonnarme P, Gillet B, Sepulchre AM, Role C, Beloeil JC, Ducrocq C (1995) Itaconate biosynthesis in *Aspergillus-terreus*. *J Bacteriol* 177(12):3573–3578
- Calam CT (1939) Studies in the biochemistry of microorganisms. XXIII. Itaconic acid, a metabolic product of *Aspergillus terreus* Thom. *Biochem J* 33:1488–1495
- Carstensen F, Klement T, Buchs J, Melin T, Wessling M (2013) Continuous production and recovery of itaconic acid in a membrane bioreactor. *Bioresour Technol* 137:179–187. <https://doi.org/10.1016/j.biortech.2013.03.044>
- Chin T, Sano M, Takahashi T, Ohara H, Aso Y (2015) Photosynthetic production of itaconic acid in *Synechocystis* sp PCC6803. *J Biotechnol* 195:43–45. <https://doi.org/10.1016/j.jbiotec.2014.12.016>
- Choudhary AQ, Pirt SJ (1966) The influence of metal-complexing agents on citric acid production by *Aspergillus niger*. *J Gen Microbiol* 43(1):71–81. <https://doi.org/10.1099/00221287-43-1-71>
- Clark DS, Ito K, Horitsu H (1966) Effect of manganese and other heavy metals on submerged citric acid fermentation of molasses. *Biotechnol Bioeng* 8(4):465–471
- Cros P, Schneider D (1993) Microbiological production of itaconic acid. United States Patent 5 231 016
- Deak E, Wilson SD, White E, Carr JH, Balajee SA (2009) *Aspergillus terreus* accessory conidia are unique in surface architecture, cell wall composition and germination kinetics. *PLoS One* 4(10):e7673. <https://doi.org/10.1371/journal.pone.0007673>
- Delidovich I, Hausoul PJ, Deng L, Pfütenreuter R, Rose M, Palkovits R (2016) Alternative monomers based on lignocellulose and their use for polymer production. *Chem Rev* 116(3):1540–1599. <https://doi.org/10.1021/acs.chemrev.5b00354>
- Durant Y (2009) Development of integrated production of polyitaconic acid from northeast hardwood biomass—NIFA project 2009–2012. Technical report, Itaconix, LLC
- Dwiarti L, Otsuka M, Miura S, Yaguchi M, Okabe M (2007) Itaconic acid production using sago starch hydrolysate by *Aspergillus terreus* TN484-M1. *Bioresour Technol* 98(17):3329–3337. <https://doi.org/10.1016/j.biortech.2006.03.016>
- Dwiarti L, Yamane K, Yamatani H, Kahar P, Okabe M (2002) Purification and characterization of cis-aconitic acid decarboxylase from *Aspergillus terreus* TN484-M1. *J Biosci Bioeng* 94(1):29–33
- Eimhjellen KE, Larsen H (1955) Mechanism of itaconic acid formation by *Aspergillus-terreus*. 2. Effect of substrates and inhibitors. *Biochem J* 60(1–4):139–147
- Fuchs G, Schlegel HG (2006) Allgemeine Mikrobiologie. Thieme Flexible Taschenbücher, Thieme Georg Verlag
- Geiser E, Przybilla SK, Friedrich A, Buckel W, Wierckx N, Blank LM, Bolker M (2016a) *Ustilago maydis* produces itaconic acid via the unusual intermediate trans-aconitate. *Microb Biotechnol* 9(1):116–126. <https://doi.org/10.1111/1751-7915.12329>
- Geiser E, Przybilla SK, Engel M, Kleineberg W, Buttner L, Sarikaya E, Hartog TD, Klankermayer J, Leitner W, Bolker M, Blank LM, Wierckx N (2016b) Genetic and biochemical insights into the itaconate pathway of *Ustilago maydis* enable enhanced production. *Metab Eng* 38:427–435. <https://doi.org/10.1016/j.ymben.2016.10.006>
- Geiser E, Wiebach V, Wierckx N, Blank LM (2014) Prospecting the biodiversity of the fungal family *Ustilaginaceae* for the production of value-added chemicals. *Fungal Biol Biotechnol* 1(1):2. <https://doi.org/10.1186/s40694-014-0002-y>
- Guevara ED, Tabuchi T (1990) Production of 2-hydroxyparaconic and itatartaric acids by *Ustilago cynodontis* and simple recovery process of the acids. *Agric Biol Chem* 54(9):2359–2365
- Guevarra ED, Tabuchi T (1990) Accumulation of itaconic, 2-hydroxyparaconic, itatartaric, and malic-acids by strains of the genus *Ustilago*. *Agric Biol Chem* 54(9):2353–2358

- Gyamerah M (1995a) Factors affecting the growth form of *Aspergillus terreus* NRRL 1960 in relation to itaconic acid fermentation. *Appl Microbiol Biot* 44(3–4):356–361
- Gyamerah MH (1995b) Oxygen requirement and energy relations of itaconic acid fermentation by *Aspergillus terreus* NRRL 1960. *Appl Microbiol Biot* 44(1–2):20–26
- Harder BJ, Bettenbrock K, Klamt S (2016) Model-based metabolic engineering enables high yield itaconic acid production by *Escherichia coli*. *Metab Eng* 38:29–37. <https://doi.org/10.1016/j.ymben.2016.05.008>
- Haskins RH, Thorn JA, Boothroyd B (1955) Biochemistry of the *Ustilaginales*. 11. Metabolic products of *Ustilago zaeae* in submerged culture. *Can J Microbiol* 1(9):749–756
- Hevekerl A (2016) Biotechnisch erzeugte Itaensäure. Cuvillier Verlag
- Hevekerl A, Kuenz A, Vorlop K-D (2014a) Filamentous fungi in microtiter plates—an easy way to optimize itaconic acid production with *Aspergillus terreus*. *Appl Microbiol Biot*:1–7 doi:<https://doi.org/10.1007/s00253-014-5743-2>
- Hevekerl A, Kuenz A, Vorlop KD (2014b) Influence of the pH on the itaconic acid production with *Aspergillus terreus*. *Appl Microbiol Biot* 98(24):10005–10012. <https://doi.org/10.1007/s00253-014-6047-2>
- Hewald S, Josephs K, Bolker M (2005) Genetic analysis of biosurfactant production in *Ustilago maydis*. *Appl Environ Microb* 71(6):3033–3040
- Holzhäuser FJ, Artz J, Palkovits S, Kreyenschulte D, Büchs J, Palkovits R (2017) Electrocatalytic upgrading of itaconic acid to methylsuccinic acid using fermentation broth as a substrate solution. *Green Chem* 19(10):2390–2397. <https://doi.org/10.1039/C6GC03153F>
- Hossain AH, Li A, Brickwedde A, Wilms L, Caspers M, Overkamp K, Punt PJ (2016) Rewiring a secondary metabolite pathway towards itaconic acid production in *Aspergillus niger*. *Microb Cell Factories* 15(1):130. <https://doi.org/10.1186/s12934-016-0527-2>
- Huang XN, Chen M, Li JJ, Lu XF (2016) Establishing an efficient gene-targeting system in an itaconic-acid producing *Aspergillus terreus* strain. *Biotechnol Lett* 38(9):1603–1610. <https://doi.org/10.1007/s10529-016-2143-y>
- Jaklitsch WM, Kubicek CP, Scrutton MC (1991) The subcellular organization of itaconate biosynthesis in *Aspergillus terreus*. *J Gen Microbiol* 137:533–539
- Jeon HG, Cheong DE, Han Y, Song JJ, Choi JH (2016) Itaconic acid production from glycerol using *Escherichia coli* harboring a random synonymous codon-substituted 5'-coding region variant of the *cadA* gene. *Biotechnol Bioeng* 113(7):1504–1510. <https://doi.org/10.1002/bit.25914>
- Jimenez-Quero A, Pollet E, Zhao M, Marchioni E, Averous L, Phalip V (2016) Itaconic and fumaric acid production from biomass hydrolysates by *Aspergillus* strains. *J Microbiol Biotechnol* 26(9):1557–1565. <https://doi.org/10.4014/jmb.1603.03073>
- Jönsson LJ, Alriksson B, Nilvebrant NO (2013) Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnol Biofuels* 6:16
- Juy MI, Orejas JA, Lucca ME (2010) Study of itaconic acid production by *Aspergillus terreus* MJL05 strain with different variable. *Rev Colom Biotechnol* 12:187–193
- Kamper J, Kahmann R, Bolker M, Ma LJ, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Muller O, Perlin MH, Wosten HAB, de Vries R, Ruiz-Herrera J, Reynaga-Pena CG, Snetselaar K, McCann M, Perez-Martin J, Feldbrugge M, Basse CW, Steinberg G, Ibeas JJ, Holloman W, Guzman P, Farman M, Stajich JE, Sentandreu R, Gonzalez-Prieto JM, Kennell JC, Molina L, Schirawski J, Mendoza-Mendoza A, Greilinger D, Munch K, Rossel N, Scherer M, Vranes M, Ladendorff O, Vincon V, Fuchs U, Sandrock B, Meng S, Ho ECH, Cahill MJ, Boyce KJ, Klose J, Klosterman SJ, Dealstra HJ, Ortiz-Castellanos L, Li WX, Sanchez-Alonso P, Schreier PH, Hauser-Hahn I, Vaupel M, Koopmann E, Friedrich G, Voss H, Schluter T, Margolis J, Platt D, Swimmer C, Gnirke A, Chen F, Vysotskaia V, Mannhaupt G, Guldener U, Munsterkötter M, Haase D, Oesterheld M, Mewes HW, Mauceli EW, DeCaprio D, Wade CM, Butler J, Young S, Jaffe DB, Calvo S, Nussbaum C, Galagan J, Birren BW (2006) Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444(7115):97–101
- Kanamasa S, Dwiarti L, Okabe M, Park EY (2008) Cloning and functional characterization of the cis-aconitic acid decarboxylase (*CAD*) gene from *Aspergillus terreus*. *Appl Microbiol Biot* 80(2):223–229. <https://doi.org/10.1007/s00253-008-1523-1>
- Kane J, Finlay A, Amann P (1945) Production of itaconic acid. United States Patent 2 385 283
- Karaffa L, Diaz R, Papp B, Fekete E, Sandor E, Kubicek CP (2015) A deficiency of manganese ions in the presence of high sugar concentrations is the critical parameter for achieving high yields of itaconic acid by *Aspergillus terreus*. *Appl Microbiol Biot* 99(19):7937–7944. <https://doi.org/10.1007/s00253-015-6735-6>
- Kawamura D, Furuhashi M, Saito O, Matsui H (1981) Japan Patent 56 137 893
- Kim J, Seo HM, Bhatia SK, Song HS, Kim JH, Jeon JM, Choi KY, Kim W, Yoon JJ, Kim YG, Yang YH (2017) Production of itaconate by whole-cell bioconversion of citrate mediated by expression of multiple cis-aconitate decarboxylase (*cadA*) genes in *Escherichia coli*. *Sci Rep* 7:39768. <https://doi.org/10.1038/srep39768>
- Kinoshita K (1932) Über die Produktion von Itaensäure und Mannit durch einen neuen Schimmelpilz, *Aspergillus itaconicus*. *Acta Phytochimica* 5:271–287
- Klement T, Büchs J (2013) Itaconic acid—a biotechnological process in change. *Bioresour Technol* 135:422–431. <https://doi.org/10.1016/j.biortech.2012.11.141>
- Kobayashi T (1971) Process for recovering itaconic acid and salts thereof from fermented broth. Japan Patent 3 621 053
- Kobayashi T (1978) Production of itaconic acid from wood waste. *Process Biochem* 13(5):15–22
- Kobayashi T, Nakamura I, Nakagawa M (1972) Process design for itaconic acid fermentation. *Proc IV IFS: Ferm Technol today*. 215–221
- Kobayashi T, Nakamura I, Nakagawa M (1973) Itaconic acid production. Japan Patent 48 092 584
- Kobayashi T, Nakamura I, Nakagawa M (1980) Itaconic acid production. Japan Patent 51 028 711
- Krull S, Eidt L, Hevekerl A, Kuenz A, Prüße U (2017a) Itaconic acid production from wheat chaff by *Aspergillus terreus*. *Process Biochem* 63:169–176. <https://doi.org/10.1016/j.procbio.2017.08.010>
- Krull S, Hevekerl A, Kuenz A, Prüße U (2017b) Process development of itaconic acid production by a natural wild type strain of *Aspergillus terreus* to reach industrially relevant final titers. *Appl Microbiol Biot* 101:4063–4072. <https://doi.org/10.1007/s00253-017-8192-x>
- Kück U, Nowrousian M, Reiß J, Hoff B, Engh I (2009) Schimmelpilze: Lebensweise, Nutzen, Schaden, Bekämpfung. Springer
- Kuenz A (2008) Itaensäureherstellung aus nachwachsenden Rohstoffen als Ersatz für petrochemisch hergestellte Acrylsäure. PhD thesis, Technical University of Braunschweig
- Kuenz A, Gallenmüller Y, Willke T, Vorlop K-D (2012) Microbial production of itaconic acid: developing a stable platform for high product concentrations. *Appl Microbiol Biot* 96(5):1209–1216. <https://doi.org/10.1007/s00253-012-4221-y>
- Kumar S, Krishnan S, Samal SK, Mohanty S, Nayak SK (2017) Itaconic acid used as a versatile building block for the synthesis of renewable resource-based resins and polyesters for future prospective: a review. *Polym Int* 66(10):1349–1363. <https://doi.org/10.1002/pi.5399>
- Lai LST, Hung CS, Lo CC (2007) Effects of lactose and glucose on production of itaconic acid and lovastatin by *Aspergillus terreus* ATCC 20542. *J Biosci Bioeng* 104(1):9–13
- Lambert RJ, Stratford M (1999) Weak-acid preservatives: modelling microbial inhibition and response. *J Appl Microbiol* 86(1):157–164

- Larsen H, Eimhjellen K (1955) The mechanism of itaconic acid formation by *Aspergillus terreus*. I. The effect of acidity. *Biochem J* 60(1):135–139
- LeafDutchDNO (2015a) Leaf Technologies and Dutch DNA Biotech enter in R&D collaboration for the development of a value added fermentation solution to produce itaconic acid. http://www.lesaffre.com/wp-content/uploads/2015/10/PR_LeafDutchDNO_EN.pdf. Accessed 22.12.2017
- LeafDutchDNO (2015b) LEAF Technologies partners with Dutch DNA to produce itaconic acid. *Int Sugar J* 117(1403):783–783
- Levinson WE, Kurtzman CP, Kuo TM (2006) Production of itaconic acid by *Pseudozyma antarctica* NRRL Y-7808 under nitrogen-limited growth conditions. *Enzyme Microb Tech* 39(4):824–827
- Li A, Pfelzer N, Zuijderwijk R, Punt P (2012) Enhanced itaconic acid production in *Aspergillus niger* using genetic modification and medium optimization. *BMC Biotechnol* 12:57
- Li A, van Luijk N, ter Beek M, Caspers M, Punt P, van der Werf M (2011) A clone-based transcriptomics approach for the identification of genes relevant for itaconic acid production in *Aspergillus*. *Fungal Genet Biol* 48(6):602–611. <https://doi.org/10.1016/j.fgb.2011.01.013>
- Li X, Zheng K, Lai C, Ouyang J, Yong Q (2016) Improved itaconic acid production from undetoxified enzymatic hydrolysate of steam-exploded corn stover using an *Aspergillus terreus* mutant generated by atmospheric and room temperature plasma. *Bioresources* 11(4):9047–9058
- Listofcompanies (2017) <http://www.listofcompaniesin.com/china/itaconic-acid/>. Accessed 22.12.2017
- Lockwood LB (1975) Production of organic acids by fermentation. In: Peppler HJ, Perlman D (eds) *Microbial technology*, vol 2. Academic Press, New York, pp 356–386
- Lockwood LB, Reeves MD (1945) Some factors affecting the production of itaconic acid by *Aspergillus terreus*. *Arch Biochem* 6(3):455–469
- López-Garzón CS, Straathof AJJ (2014) Recovery of carboxylic acids produced by fermentation. *Biotechnol Adv* 32(5):873–904. <https://doi.org/10.1016/j.biotechadv.2014.04.002>
- Maassen N, Panakova M, Wierckx N, Geiser E, Zimmermann M, Bölker M, Klinner U, Blank LM (2014) Influence of carbon and nitrogen concentration on itaconic acid production by the smut fungus *Ustilago maydis*. *Eng Life Sci* 14:129–134. <https://doi.org/10.1002/elsc.201300043>
- Magalhães AI, de Carvalho JC, Ramírez ENM, Medina JDC, Soccol CR (2016a) Separation of itaconic acid from aqueous solution onto ion-exchange resins. *J Chem Eng Data* 61(1):430–437. <https://doi.org/10.1021/acs.jced.5b00620>
- Magalhães AI Jr, de Carvalho JC, Medina JD, Soccol CR (2016b) Downstream process development in biotechnological itaconic acid manufacturing. *Appl Microbiol Biotechnol* 101:1–12. <https://doi.org/10.1007/s00253-016-7972-z>
- Marked Report (2015) Transparency market research, Market Report, Itaconic Acid
- Mattey M (1992) The production of organic acids. *Crit Rev Biotechnol* 12(1–2):87–132
- McCoy M (2015) European biotech eye itaconic acid. *Chem Eng News* 93(41):16–17 Concentrates
- Monaghan RL, Alberts AW, Hoffman CH, Albers-Schonberg G (1981) Hypocholesteremic fermentation products and process of preparation. United States Patent 4 294 926
- Nelson GEN, Traufler DH, Kelley SE, Lockwood LB (1952) Production of itaconic acid by *Aspergillus terreus* in 20-Liter fermentors. *Ind Eng Chem* 44(5):1166–1168. <https://doi.org/10.1021/ie50509a062>
- Okabe M, Lies D, Kanamasa S, Park EY (2009) Biotechnological production of itaconic acid and its biosynthesis in *Aspergillus terreus*. *Appl Microbiol Biot* 84(4):597–606
- Otten A, Brocker M, Bott M (2015) Metabolic engineering of *Corynebacterium glutamicum* for the production of itaconate. *Metab Eng* 30:156–165. <https://doi.org/10.1016/j.ymben.2015.06.003>
- Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. *Bioresour Technol* 74(1):25–33
- Papagianni M (2004) Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnol Adv* 22(3):189–259
- Park YS, Ohta N, Okabe M (1993) Effect of dissolved-oxygen concentration and impeller tip speed on itaconic acid production by *Aspergillus terreus*. *Biotechnol Lett* 15(6):583–586
- Pedroso GB, Montipó S, Mario DAN, Alves SH, Martins AF (2017) Building block itaconic acid from left-over biomass. *Biomass Convers Biorefin* 7(1):23–35. <https://doi.org/10.1007/s13399-016-0210-1>
- Petrucchioli M, Pulci V, Federici F (1999) Itaconic acid production by *Aspergillus terreus* on raw starchy materials. *Lett Appl Microbiol* 28(4):309–312
- Pfeifer VF, Vojnovich C, Heger EN (1952) Itaconic acid by fermentation with *Aspergillus terreus*. *Ind Eng Chem* 44(12):2975–2980. <https://doi.org/10.1021/ie50516a055>
- Reddy CSK, Singh RP (2002) Enhanced production of itaconic acid from corn starch and market refuse fruits by genetically manipulated *Aspergillus terreus* SKR10. *Bioresour Technol* 85(1):69–71
- Robert T, Friebel S (2016) Itaconic acid - a versatile building block for renewable polyesters with enhanced functionality. *Green Chem* 18:2922–2934. <https://doi.org/10.1039/C6GC00605A>
- Roehr M, Kubicek CP, Kominek J (1996). Citric acid, in *Biotechnology: Products of primary metabolism*, Volume 6, Second Edition (eds H.-J. Rehm and G. Reed), pp. 307–345. Wiley-VCH Verlag GmbH
- Rychtera M, Wase DAJ (1981) The growth of *Aspergillus terreus* and the production of itaconic acid in batch and continuous cultures. The influence of pH. *J Chem Technol Biotechnol* 31:509–521. <https://doi.org/10.1002/jctb.503310168>
- Saha BC (2017) Emerging biotechnologies for production of itaconic acid and its applications as a platform chemical. *J Ind Microbiol Biot* 44(2):303–315
- Saha BC, Kennedy GJ (2017a) Mannose and galactose as substrates for production of itaconic acid by *Aspergillus terreus*. *Lett Appl Microbiol* 65(6):527–533. <https://doi.org/10.1111/lam.12810>
- Saha BC, Kennedy GJ (2017b) Ninety six well microtiter plate as microbioreactors for production of itaconic acid by six *Aspergillus terreus* strains. *J Microbiol Methods* 144:53–59. <https://doi.org/10.1016/j.mimet.2017.11.002>
- Saha BC, Kennedy GJ, Qureshi N, Bowman MJ (2017) Production of itaconic acid from pentose sugars by *Aspergillus terreus*. *Biotechnol Prog* 33(4):1059–1067. <https://doi.org/10.1002/btpr.2485>
- Sayama A, Kobayashi K, Ogoshi A (1994) Morphological and physiological comparisons of *Helicobasidium mompa* and *H. purpureum*. *Mycoscience* 35(1):15–20
- Schute K, Detoni C, Kann A, Jung O, Palkovits R, Rose M (2016) Separation in biorefineries by liquid phase adsorption: itaconic acid as case study. *ACS Sustain Chem Eng* 4(11):5921–5928. <https://doi.org/10.1021/acssuschemeng.6b00096>
- Shin WS, Lee D, Kim S, Jeong YS, Chun GT (2013) Application of scale-up criterion of constant oxygen mass transfer coefficient (kLa) for production of itaconic acid in a 50 L pilot-scale fermentor by fungal cells of *Aspergillus terreus*. *J Microbiol Biotechnol* 23(10):1445–1453
- Specht R, Aurich A, Kreyß E, Barth G, Bodinus C (2014) Verfahren zur biotechnologischen Herstellung von Itaconsäure. DE 102008011854 B4
- Steiger MG, Blumhoff ML, Mattanovich D, Sauer M (2013) Biochemistry of microbial itaconic acid production. *Front Microbiol* 4: Article 23

- Strelko CL, Lu WY, Dufort FJ, Seyfried TN, Chiles TC, Rabinowitz JD, Roberts MF (2011) Itaconic acid is a mammalian metabolite induced during macrophage activation. *J Am Chem Soc* 133(41):16386–16389
- Tabuchi T, Sugisawa T, Ishidori T, Nakahara T, Sugiyama J (1981) Itaconic acid fermentation by a yeast belonging to the genus *Candida*. *Agric Biol Chem* 45(2):475–479
- Tate BE (1981) Itaconic acid and derivatives. Grayson MEckroth E (eds) *Kirk-Othmer Encycl Chem Technol* 3:865–873
- Tevez G, Bencina M, Legisa M (2010) Enhancing itaconic acid production by *Aspergillus terreus*. *Appl Microbiol Biot* 87(5):1657–1664. <https://doi.org/10.1007/s00253-010-2642-z>
- Tippkötter N, Duwe AM, Wiesen S, Sieker T, Ulber R (2014) Enzymatic hydrolysis of beech wood lignocellulose at high solid contents and its utilization as substrate for the production of biobutanol and dicarboxylic acids. *Bioresour Technol* 167:447–455. <https://doi.org/10.1016/j.biortech.2014.06.052>
- Tomlinson N, Campbell JJR, Trussell PC (1950) The influence of zinc, iron, copper, and manganese on the production of citric acid by *Aspergillus niger*. *J Bacteriol* 59(2):217–227
- Tsao G, Ouyang P, Chen J (2010) *Biotechnology in China II: chemicals, energy and environment*. Advances in biochemical engineering/biotechnology. Springer Berlin Heidelberg
- van der Straat L, Tamayo-Ramos J, Schonewille T, de Graaff L (2013) Overexpression of a modified 6-phosphofructo-1-kinase results in an increased itaconic acid productivity in *Aspergillus niger*. *AMB Express* 3(1):57
- van der Straat L, Vernooij M, Lammers M, van den Berg W, Schonewille T, Cordewener J, van der Meer I, Koops A, de Graaff LH (2014) Expression of the *Aspergillus terreus* itaconic acid biosynthesis cluster in *Aspergillus niger*. *Microb Cell Factories* 13:11. <https://doi.org/10.1186/1475-2859-13-11>
- Wang JH, Tsai SH, Teng K (2012) Producing itaconic acid in yeast using glycerol as the substrate. United States Patent 8(192):965
- Weastra sro (2013) Wp 8.1, determination of market potential for selected platform chemicals: itaconic acid, succinic acid, 2,5-furandicarboxylic acid. Technical report, Bioconcept
- Welter K (2000) *Biotechnische Produktion von Itaconsäure aus nachwachsenden Rohstoffen mit immobilisierten Zellen*. PhD thesis, Technical University of Braunschweig
- Werpy T, Petersen G, Aden A, Bozell J, Holladay J, White J, Manheim A, Eliot D, Lasure L, Jones S (2004) Top value added chemicals from biomass: volume I—results of screening for potential candidates from sugars and synthesis gas. Technical report, Department of Energy Washington DC
- Willke T, Vorlop KD (2001) Biotechnological production of itaconic acid. *Appl Microbiol Biot* 56(3–4):289–295
- Wu X, Liu Q, Deng Y, Li J, Chen X, Gu Y, Lv X, Zheng Z, Jiang S, Li X (2017) Production of itaconic acid by biotransformation of wheat bran hydrolysate with *Aspergillus terreus* CICC40205 mutant. *Bioresour Technol* 241:25–34. <https://doi.org/10.1016/j.biortech.2017.05.080>
- Yahiro K, Shibata S, Jia SR, Park Y, Okabe M (1997) Efficient itaconic acid production from raw corn starch. *J Ferment Bioeng* 84(4):375–377
- Yahiro K, Takahama T, Park YS, Okabe M (1995) Breeding of *Aspergillus-terreus* mutant TN-484 for itaconic acid production with high-yield. *J Ferment Bioeng* 79(5):506–508
- Zambanini T, Hartmann SK, Schmitz LM, Buttner L, Hosseinpour Tehrani H, Geiser E, Beudels M, Venc D, Wandrey G, Buchs J, Schwarzlander M, Blank LM, Wierckx N (2017a) Promoters from the itaconate cluster of *Ustilago maydis* are induced by nitrogen depletion. *Fungal Biol Biotechnol* 4:11. <https://doi.org/10.1186/s40694-017-0040-3>
- Zambanini T, Hosseinpour Tehrani H, Geiser E, Merker D, Schlee S, Krabbe J, Buescher JM, Meurer G, Wierckx N, Blank LM (2017b) Efficient itaconic acid production from glycerol with *Ustilago vetiveriae* TZ1. *Biotechnol Biofuels* 10:131. <https://doi.org/10.1186/s13068-017-0809-x>