

Fermentative Itaconic Acid Production

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Abstract

Itaconic acid is an organic acid that is used as a platform chemical for the production of various value added chemicals such as poly-itaconic acid, resins biofuel components, ionomer cements etc. Itaconic acid and its derivatives have wide applications in the textile, chemical and pharmaceutical industries. The depletion of fossil fuels and the need for sustainable development require that fermentative itaconic acid production replace petroleum-based methods of itaconic acid production. Various microorganisms have been employed in itaconic acid fermentations, with the most prolific producer being *Aspergillus terreus*. Over 80 g/L itaconic acid has been produced in fermentations using glucose. However, there is an increasing interest in the utilization of lignocellulosic materials for itaconic acid production due to the concern of food security. This review summarizes the latest progress in fermentative itaconic acid production with emphasis on the different species and strains of microorganisms used, substrate types and fermentation conditions. The current industrial applications of itaconic acid and its potential use as a drop-in or novel substitute monomer to replace petroleum-based chemicals were also extensively explored. Recent trends in itaconic acid research summarized in this review paper indicate that itaconic acid can be produced cost effectively from sustainable raw materials and have the potential to replace petro-based chemicals in various applications.

Keywords: Itaconic acid; Fermentation; Renewable; *Aspergillus terreus*; Biosynthesis; Platform chemicals

Introduction

Currently, crude oil, natural gas and coal are still the primary raw materials for the production of chemicals. The increasing concern on sustainability, environmental conservation and energy shortage drives the search for viable, renewable and environmental friendly alternatives to replace fossil resources as raw material for the production of important chemicals. Plant biomass is considered to be the most feasible alternative as it is a sustainable resource and does not liberate fossilized carbon. Many chemicals such as succinic acid [1], 1,3-propanediol [2] and ethanol [3,4] which were hitherto made from petroleum refining are now being successfully produced from renewable biomass.

Among these chemicals, Itaconic Acid (IA) is an important platform chemical which has a wide range of actual and potential applications. It can be used to replace a wide range of petroleum based chemicals, e.g. acrylic acid, which will reduce dependence on petroleum and the attendant deleterious environmental effects. In spite of this, it only occupies a niche market because of its high cost relative to acrylic acid and other alternatives [5] thus limiting its use to low volume markets. It is mainly produced by the fermentation of sugars with *Aspergillus terreus* with limited success with bacterial and yeast fermentations. This review discusses the physico-chemical properties of IA, current and potential uses of itaconic acid, the microorganisms used in IA synthesis, the fermentation processes, conditions and future perspectives in itaconic acid applications.

Physico-chemical properties

Itaconic acid is a naturally occurring unsaturated 5-C dicarboxylic acid which is also known as methylenesuccinic acid or methylenebutanedioic acid [6]. Itaconic acid was first described by Baup in 1836 when he discovered it as a product of citric acid distillation. Itaconic acid has the stoichiometric formula $C_5H_6O_4$ and a molar weight of 130.1 g/mol. It exists as white to light beige crystals with a density of 1.573 g/mL at 25°C, a melting point of 165-168°C and a flash point of 268°C [6]. It dissolves in water up to 80.1 g/L at 20°C which makes it quite easy to purify by crystallization [7]. In a recent study IA was also found to dissolve well in several alcohols including methanol, 2-propanol and ethanol with the solubility increasing with temperature [8]. Itaconic acid is also readily biodegradable in nature.

Itaconic acid is valuable as a monomer because of its unique chemical properties, which derive primarily from its methylene group and its possession of two carboxylic acid groups. Itaconic acid is able to take part in addition polymerization, giving polymers with many free carboxyl groups that confer advantageous properties on the polymer. It can either be self-polymerised or can act as a co-monomer with other monomers to form heteropolymers [9]. It has two protonation states with pKa values of 3.85 - 5.45 and with a degree of reduction of 3.6, it is just a little more oxidised than glucose with a value of 4.0 [10]. Itaconic acid is about twice as acidic as acrylic acid and more reactive than maleic and fumaric acids which are potential monomeric substitutes [11].

Itaconic acid readily forms a range of metallic salts and diesters such as dimethyl itaconate and di-n-butyl itaconate both of which are available commercially. Itaconic anhydride may be used for the preparation of mono esters such as monomethyl itaconate or react with amines to yield N-substituted pyrrolidones with actual or

proposed uses in greases, detergents, shampoos, herbicides and pharmaceuticals. A condensate of lauric acid and aminoethylethanolamine reacts with IA to give an imidazoline derivative which is an active ingredient in shampoos [7]. Recently two new itaconic acid derivatives (-)-9-hydroxyhexylitaconic acid, and (-)-9-hydroxyhexylitaconic acid-4-methyl ester were discovered as metabolites of *Aspergillus aculeatus* CRI322-03 [12].

Applications and market

Itaconic acid and its polymers are currently utilized in numerous applications as drop-in or novel substitute monomer (Figure 1), where they sometimes confer favourable characteristics on the end product which makes it superior to the conventional substitutes. Common end products of IA polymerization reactions include polyitaconic acid (PIA) and styrene-butadiene rubber (SBR) latex made from the polymerization of styrene, butadiene and itaconic acid. Itaconic acid can potentially replace acrylic acid for use in the production of superabsorbent polymers with improved properties, and maleic anhydride which is currently used in the production of Unsaturated Polyester Resins (UPR); while PIA can replace sodium tripolyphosphate (STPP) used in detergents. Acrylate latexes supplemented with IA can be used as non-woven fabric binders, and a copolymer of IA and acrylonitrile is also easier to dye than many other polymers while carpets containing IA as a sizing agent have enhanced resistance to abrasion [13].

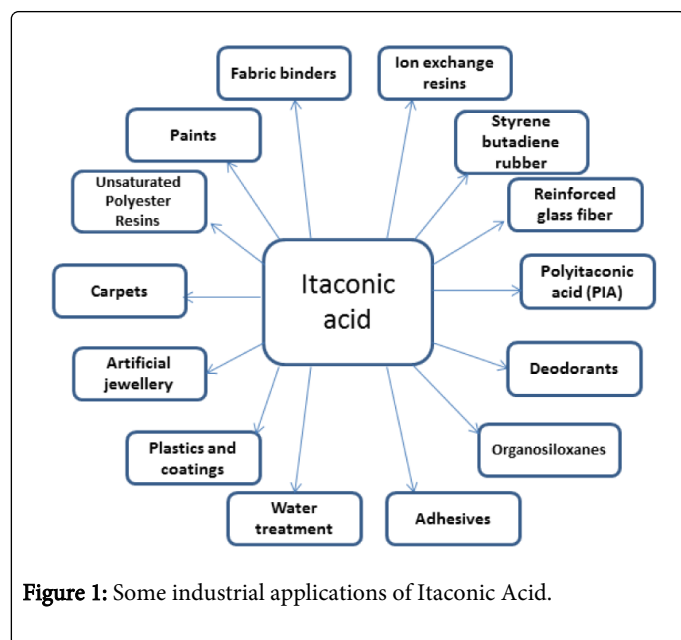


Figure 1: Some industrial applications of Itaconic Acid.

Itaconic acid is also used in the manufacture of emulsion paints, where it improves the adhesion of the paint. When IA is added at a level of 5% in acrylic resins, it imparts to the resins the ability to hold printing inks [14]. In plastics and coatings, a 1-5% addition confers on the product benefits such as a light colour, ease of painting and separation, water-fastness and antiseptic properties. In the medical/pharmaceutical industry, IA is used as a hardening agent in organosiloxanes for use in contact lenses; in binders for use in diapers and feminine napkins, in the production of glass ionomer cement a biocompatible cement used in dentistry [15,16]; while Tomic et al. [10] synthesised hydrogels, based on 2-hydroxyethyl methacrylate (HEMA) and IA copolymers which promoted wound healing and prevented

bacterial contamination. In addition, esters of partly substituted itaconic acid possess analgesic properties while some display plant-growth related activities [17]. It is also used in water treatment technologies [5] and in the synthesis of potential biofuel additives such as 3-methyltetrahydrofuran and 2-methylbutanediol [18,19].

In a relatively novel application by Pascale et al. [20], IA was identified as a monomer with high affinity to deoxynivalenol (DON) a mycotoxin of *Fusarium sp.* found in cereals such as sorghum and maize [21] The authors were able to bind up to 90% of DON in water and water-polyethyleneglycol and 70% in pasta extracted with water and phosphate buffered saline [20]. They thus demonstrated the use of an IA polymer which can be used as a low-cost material for clean-up and pre-concentration of DON from food materials, allowing HPLC determination of the toxin around the regulatory levels.

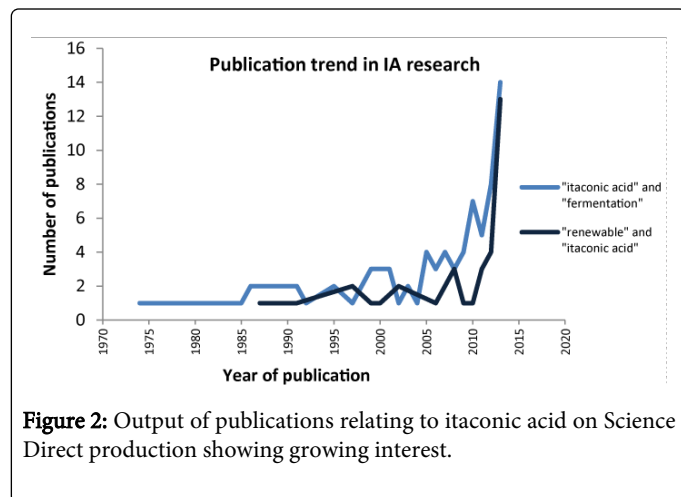
In a report compiled by Weastra [13], it was estimated that the annual global production of IA was over 41,000 tonnes worth \$74.5 million in 2011 and projected to surpass 50,000 tonnes amounting to over \$567 million by 2020. China, USA, France and Japan were the major IA producing countries in the world. When Cargill and Pfizer in the U.S.A, and the French company Rhodia stopped production of IA, China became the largest producer with companies like Qingdao Kehai Biochemistry having an annual output capacity of 20,000 tonnes. A host of factors have led to this decline in IA production and notable among them are the slow rate of development of viable end use applications, its relatively high price and stable petroleum prices which will make its use to replace the relatively cheaper petroleum-based polyacrylamide uneconomical. At the current price of between \$1800-2000/ton it is not economically favourable to use IA in many applications, however if the price can fall below \$1,500/ton, a complete replacement of petroleum-based polyacrylic acid with IA will be worth over \$11 billion annually [5,13].

History of itaconic acid production

Itaconic acid was historically produced by various chemical methods: (1) Destructive distillation of citric acid, and this was the main method of producing it prior to the 1960s; (2) Heating the calcium aconitate solution produced in the cane sugar refining process; (3) The patented Montecatini method involving propargyl chloride; (4) The oxidation of mesityl oxide and subsequent isomerisation of the formed citric acid; and (5) The oxidation of isoprene. Maleic anhydride is also used in the production of IA but the process has not yet achieved industrialization. None of these (or other) processes compete favourably with the fermentative production process [22], and IA is now almost entirely produced by fermentation of sugars by *Aspergillus terreus* [23].

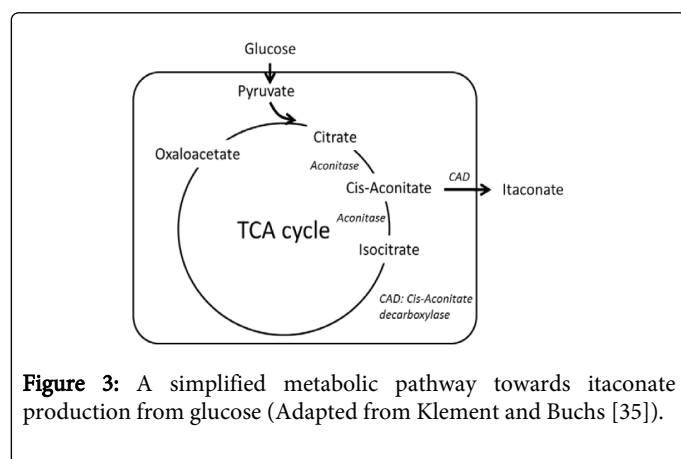
Itaconic acid was first discovered to be a biological metabolite when it found to be produced by a strain of *Aspergillus* which was subsequently named *Aspergillus itaconicus* [24] while Calam et al. [25] discovered that *A. terreus* can also accumulate IA in even greater amounts than *A. itaconicus*. The Northern Regional Research Laboratory (NRRL) of the United States Department of Agriculture (USDA) screened several wild type strains and identified *A. terreus* NRRL 1960, as the most prolific IA-producing strain [26] which then went on to become the most published strain. At the same institute, preliminary attempts were also made to develop a biotechnical process for IA production [27]. Later, optimised industrial processes were established providing the limited market with IA.

The main developments in IA production occurred within the next two decades. Based on the number of scientific publications recorded, it can be inferred that the interest in IA production then declined. Following the release of a report published by the United States' Department of Energy (DoE) which lists IA as one of 12 promising chemicals obtainable from biomass [28], there was a renewed interest in its production (Figure 2).



Itaconic acid biosynthesis

The process of the biosynthesis of itaconic acid has been a subject of academic discourse and is only recently being agreed upon. In 1931, Kinoshita [24] suggested that IA was produced in *A. terreus* by the decarboxylation of aconitate. This was supported by tracer studies of Bentley and Thiesse [29-31] who discovered the presence of a cis-aconitate decarboxylase (CAD) enzyme which was proposed to catalyse the production of IA. Dwiarti et al. [32] showed that the pathway via aconitase and CAD was most likely (Figure 3) and they isolated and characterised the CAD enzyme. In 2008, the CAD gene was identified by Kanamasa and co-workers [33] who used it to transform *Saccharomyces cerevisiae*, proving that the gene was indeed responsible for the production of the CAD enzyme, and that the CAD enzyme catalysed the production of IA. Li et al. [34] transformed *A. niger* cells with the CAD gene and were able to initiate IA production in *A. niger*. The review by Klement and Buchs [35] thoroughly discusses the details of IA biosynthesis and regulation in *A. terreus*. Figure 3 shows a simplified pathway for IA production in *A. terreus*.



Itaconic acid fermentative production

Submerged fermentation

The conditions for the production of IA by *Aspergillus terreus* by submerged fermentation (SF) are similar to those of citric acid production by *A. niger*. These conditions include the availability of an excess of readily metabolizable carbon source, high levels of dissolved oxygen, limiting amounts of metal ions and an ammonium-based nitrogen source.

Microorganisms

A number of microorganisms have been screened and studied for itaconic acid production. Since it was observed that *A. terreus* produces more IA than *A. itaconicus* the search for the ideal industrial organism for IA production has continued and several attempts have been made to find microorganisms with improved characteristics including greater yields, higher tolerance to shear stress, and/or tolerance to higher end product concentration.

Aspergillus terreus Strains

The most widely used strain in IA production is a native strain known as *A. terreus* NRRL 1960, which is also stored in various culture collections under the names CBS 116.46, DSM 826, IAM 2054, IFO 6123, IMI 44243, QM 6856 and WB 1960 [36]. This strain was recorded to produce up to 91 g/L IA from glucose [37]. *Aspergillus terreus* IMI 282743 is another native strain which yielded 5.76 g/L IA from palm oil mill effluent [38] while IA concentrations of around 54 g/L on a medium containing 15% glucose have been reported from wild-type Egyptian strains [39]. In spite of these yields recorded in literature, the need for even higher yields has necessitated strain improvement as a result of which most high-yielding strains available in literature today are modified strains.

To improve IA production, both strain mutagenesis and genetic modification have been explored on *A. terreus* strains. Yahiro et al. [40] successively mutated a wild type *A. terreus* strain IFO6365 and isolated a strain designated *A. terreus* TN484-M1, which produced up to 82 g/L IA within 6 days of fermentation. The transcription of CAD1 (the gene encoded cis-aconitic acid decarboxylase) in the mutant strain was five times stronger than that of IFO6365, which was believed to account for the improved performance [33]. Dwiarti et al. [41] explored the IA production from sago starch using the same strain and around 48.2 g/L IA was obtained.

Reddy and Singh [42] mutated *A. terreus* SKR10, a native strain, using various chemicals or ultraviolet mutagens separately and in combination. They obtained two improved mutants: *A. terreus* N45 which produced 50 g/L IA from corn starch, and *A. terreus* UNCS1 which produced 32 g/L IA from fruit waste extracts. In comparison, the parent strain only synthesized 31 g/L and 20 g/L IA from the two substrates, respectively.

A wild type strain of *A. terreus* was modified by incorporating a modified pfkA gene from *A. niger* into its genome [43]. This modified pfkA gene encoded for a shorter and more active 6-phospho-fructo-1-kinase enzyme fragment. They obtained transformants that accumulated higher amounts of IA than the native strain, albeit after a longer lag phase. The IA obtained from the best transformant A729 was 45.5 g/L, which was more than twice of that achieved by the parent strain (21.35 g/L).

Other microorganisms

Various organisms possess various inherent benefits which they can impart on the IA fermentation process. The ideal organism will be one with yields as high as (or even surpass) *A. terreus*, is amenable to genetic manipulation like *E. coli*, can be easily handled like yeasts and will have minimum by-product formation. These desired qualities can be imparted into a host organism through genetic manipulation and a few attempts have yielded encouraging results.

Apart from *A. terreus* and *A. itaconicus*, several other fungi have also been used for the IA production. It was detected in the fermentation broth of an *Ustilago zaeae* strain while screening several *Ustilago* strains for the production of *ustilagic* acid [44]. Later, the Iwata Corporation (Japan) recorded a concentration of 53 g/L IA by *U. maydis* using glucose as substrate [45,46]. Levinson et al. [47] obtained 30 g/L of IA from one strain of the basidiomycete *Pseudozyma antarctica* in shake flask cultures. Similarly, a yeast strain identified as *Candida* was obtained after screening and subsequent mutation, which produced about 53 g/L IA in 5 days [48].

Tkacz and Lange [49] proposed a strategy of introducing the cis-aconitate decarboxylase gene into a citric acid producing strain e.g. *A. niger* for the IA production. This was investigated by Li et al. [34,50] who successfully developed *A. niger* transformants and optimized medium conditions, leading to 0.13 g/L IA production. Similarly, an increase in IA yields up to a factor of 14-24 was obtained by targeting the key enzymes in IA synthesis, aconitase and CAD, to the right cellular compartments in *A. niger* [51]. Similarly *Escherichia coli* could be genetically modified for IA synthesis [52].

Carbon Source/Substrate

Sugars/Starch

Although *A. terreus* grows well on most mono- and disaccharides, it can convert only a few of them into IA [53]. Glucose is an easily metabolizable substrate for *A. terreus* and is most widely used in IA production [42,44,46]. Sucrose is also a commonly used substrate. Arabinose, xylose and lactose have also been tested for IA production [54,55], but the yields were low (18% from arabinose, 31% from xylose and less than 1% from lactose). Starches from sago, sorghum, sweet

potato, wheat, potato, and cassava have been successfully used to produce IA with varying degrees of success and these results are summarized in Table 1. These starches however have food uses, thus their use in IA production can be considered to conflict with food applications.

Agro-Wastes and orgAnic acid

With the current price of glucose ranging from \$0.35- \$0.60/kg and sucrose from \$0.45- \$0.72/kg, the use of these sugars as substrates for IA production contributes significantly the production costs. The use of cheaper substrates, such as agro-wastes, could potentially bring down the cost of IA production. Table 1 presents some examples of IA formation using agriculture waste streams, such as jatropha seed cake, molasses and corn syrup. Agro-wastes are usually pre-treated by one or more of a variety of methods elucidated in various reports [18,56,57].

The major challenges posed by the use of these complex substrates are: (1) Their very variable composition across substrates and even within different batches of the same substrate. (2) The yields from crude substrates are generally lower than those from sugars. (3) The presence of potentially harmful chemicals in the substrate which occur naturally, or are formed during pre-treatment processes may affect the growth and output of the fermenting organism, further lowering yields. (4) Additionally, impurities in the end product further raises cost in the form of expensive purification requirements.

Interestingly, citric acid has also been used as a precursor in IA biosynthesis. With the price of citric acid being significantly less than that of IA, this method may prove to be economical [58].

Minerals and Nutrients

Like many other fungal fermentations, IA fermentation is sensitive to the concentrations of trace metals and nitrogen sources. Itaconic acid yield is significantly improved by the presence of nitrogen and trace amounts of Zn²⁺ and Fe²⁺ ions [26] while phosphate ion should be limited once mycelial growth is established to prevent carbon diversion into further mycelia production. The presence and quantities of these ions in complex substrates are usually outside the optimum and can negatively influence yields.

| Microorganism | Substrate | Temp. (c) | Time (days) | pH | Aeration (rpm) | Concentration (g/l) | Reference |
|---|--------------------|-----------|-------------|-----|----------------|---------------------|-----------|
| <i>A. terreus</i> | Jatropha cake | R.T | 9 | 1.5 | 400 | 48.7 | [59] |
| <i>A. terreus</i> CECT 20365 | Olive & beet waste | 30 | 5 | 5.5 | N/A | 44 | [60] |
| <i>A. terreus</i> XL-6 | Potato starch | 36.7 | 5 | 2.4 | 197 | N/A | [61] |
| <i>A. terreus</i> | Glucose, glycerol | 37 | 6 | 4.5 | 200 | 26.9&30.2 | [62] |
| <i>A. terreus</i> DSM23081, NRRL1960, NRRL 1963 | Glucose | 33 | 7 | 3.1 | 120 | 87-91 | [37] |
| <i>A. terreus</i> MJL05 | Glycerol | 30 | 8 | 2.4 | N/A | 27.6 | [63] |
| <i>A. terreus</i> SKR10 | Sago starch | 40 | 6 | 2.0 | 295 | 48.2 | [41] |
| <i>A. terreus</i> | Jatropha cake | 32 | 5 | 3.5 | 200 | 24.5 | [64] |
| <i>A. terreus</i> 282743 | POME | 30 | 5 | 5.8 | 150 | 5.76 | [38] |

| | | | | | | | |
|-------------------------------|---------------------|----|-------|---------|------|------|------|
| <i>A. terreus</i> | Glucose | | 7 | 3.0 | 150 | 23.5 | [65] |
| <i>A. terreus</i> M-8 | Starch hydrolysate | 35 | 4 | 2.5-2.8 | N/A | 55 | [66] |
| <i>A. terreus</i> | Citric acid | 30 | 2 | 6.4 | 200 | N/A | [58] |
| <i>A. terreus</i> NRRL1960 | Various starches | 35 | 6 | 3.4 | 500 | 18.4 | [55] |
| <i>A. terreus</i> NRRL1960 | Sucrose | 30 | 3 | - | 150 | 59 | [26] |
| <i>A. terreus</i> TN-484-M1 | Glucose | 37 | 6 | 2.0 | 220 | 82 | [40] |
| <i>A. terreus</i> TTK200-5-1 | | 37 | 14 | 3.1 | 200 | 51 | [67] |
| <i>A. terreus</i> TTK 200-5-3 | Sucrose | 37 | 14 | 3.0 | 200 | 13.3 | [68] |
| <i>A. terreus</i> | Glucose and Sucrose | 30 | 14 | 3.5 | N/A | 54 | [39] |
| <i>A. niger</i> | Glucose | 33 | 10-13 | | 180 | 1.4 | [51] |
| <i>A. niger</i> | Glucose | 33 | 5-6 | 2.3 | N/A | 2.5 | [50] |
| <i>Ustilago maydis</i> | Glucose | 34 | 5 | 3.0 | 180 | 29 | [69] |
| <i>Ustilago maydis</i> MB215 | Glucose | 30 | - | 6.8 | 300 | 20 | [18] |
| <i>Pseudozyma antarctica</i> | Sugars | 28 | 10 | 5.0 | 1000 | 30 | [47] |

Table 1: Fermentation parameters and outputs for Itaconic Acid production.

Fermentation Time and Temperature

A wide range of fermentation times have been studied for IA fermentation ranging from two to 14 days [58,67]. The optimum production time has however been reported to be seven days by many authors [70]. The reported temperatures for IA production also vary, but it is optimally kept at around 37°C [22]. Successful attempts have been made to raise this optimum and a five-fold increase in the IA yields of a parent strain were observed in a mutant strain at 40°C [71].

Dissolved Oxygen/Aeration

Itaconic acid fermentation is strictly aerobic. The effects of dissolved oxygen (DO) and agitation speed were evaluated by Park et al. [72]. It was observed that even though the overall yield increased with DO, the yield per unit of glucose consumed was highest when DO was about 20% of the saturation point at an impeller speed of 0.94 m/s (equivalent to an agitation speed of 320 rpm). Rychtera and Wase [73] found that the optimum aeration rate was at an impeller speed of 0.71 m/s while Riscaldati et al. [74] reported that IA production increased with the increase of agitation rate up to 400 rpm (an impeller speed of 1.57 m/s).

Even a temporary cessation of aeration such as during the manual sampling process in shake-flask fermentation can irreversibly damage the mycelia and effectively halt fermentation [75]. Lin et al. [76] reported the integration of *Vitreoscilla* haemoglobin gene (vgb) which reduced the impact of such short interruption in aeration during fermentation with some success. The interruption resulted in a decrease of only 39.2% in the IA production rate of the transformant pAN-vgb-2 compared to a 51.2% decrease in *A. terreus* M8, the native strain. This can be ascribed to the over 8-fold increase in oxygen uptake in the haemoglobin-transformed strain.

The optimum aeration rates for IA production reported in literature vary widely. There is thus a need to determine the appropriate aeration

needs for specific fermentation systems in order to avoid either sheer stress on the mycelia caused by excessive impeller speeds or poor oxygen transfer rate at lower speeds. Further increase in aeration rates would lead to an enhanced cell growth, but this may not justify the extra cost incurred from the increased aeration rate.

Immobilization

IA biosynthesis using immobilized microorganisms have also been investigated. The first matrix used in IA production was polyacrylamide gel [77]. Since then, various other matrices have been used including a porous disk reactor system, silica-based material, alginate, polyurethane cubes, and structural fibrous network of papaw trunk wood [27,65,67]. Vassilev et al. [61] reported that the pore size of the polyurethane foam carrier did not affect the loading rate of *A. terreus* and obtained an average yield of 15.1 g/L IA over five cycles with the carrier.

Solid state fermentation

Itaconic acid has been successfully produced using solid state fermentation (SSF). Agricultural wastes are the best substrates for SSF and have been used to successfully produce a number of important metabolites. The agro-waste may serve solely as mechanical support or anchor for the organism in which case it may be supplemented with additional nutrients, or it may contain most of the nutrients required for the fermentation. Vassilev et al. [60] produced 44 g/L IA from dried olive wastes and beet pressmud using *Aspergillus terreus* CECT 20365, while a yield of 55 g/L IA was obtained in a patented SSF method utilizing a mutant, *A. terreus* M8, on sugar solutions or starch hydrolysate adsorbed on sugarcane pressmud [66].

Moisture is a very important factor in SSF with an optimum level of 65%-70% reported for IA production from agricultural wastes [60]. Moisture levels below this optimum results in low solubility of

nutrients and decreased the swelling of the beet pressmud used as substrate while at levels above 70% the equilibrium of air:solids:water was disrupted, thus negatively affecting the porosity and particle aggregation of the substrate.

Since the optimum temperatures for IA fermentation are slightly higher than ambient, there is a need to heat the substrate bed to the optimum temperature to ensure spore germination and product formation. Aeration will serve the dual purpose of providing oxygen for the cells and recirculating the heat to avoid hotspots.

It is widely believed that SSF can provide better or similar yields as obtained in submerged fermentation in many biochemical production processes. In theory, as a strictly aerobic process which occurs at elevated temperatures and utilises a filamentous fungus, the IA production process appears to be ideally amenable to SSF conditions. Recently, there is growing interest in SSF, especially using agriculture waste as substrates. However, the fermentation conditions still need further optimization.

Downstream processing and product recovery

Itaconic acid is relatively easy to recover because it crystallizes easily. Upon the exhaustion of fermentable sugars, the fermentation liquor is filtered to remove suspended solids and mycelia. The liquor is then concentrated by evaporating to about a fifth of its original volume and then crystallized. These crystals are then washed and recrystallized to remove impurities [14]. Itaconic acid can also be precipitated as salts of metals such as calcium or lead [78]. Compared to the calcium salt, the precipitation of IA as a lead salt has the advantage of not requiring a pre-concentration of the broth, as it is almost insoluble in water. Lead is preferable to calcium salt precipitation because the lead salt of IA is almost insoluble in water being more soluble than for example calcium citrate, and thus cannot be used as readily as the latter in the process of IA purification by precipitation. In situ product removal has great potential especially in high-yielding fermentations to prevent end-product inhibition. Although recovery of itaconic acid from model solutions using electrodialysis has been tested [79], the membrane separation as a means of IA recovery is in its infancy and limited information is available.

Future perspectives

Itaconic acid has been gaining interest over the years for its potential application as an important bio-based platform chemical. The biggest market potential for itaconic acid is in new applications as unsaturated polyester resins, detergents builders and for production of methyl methacrylate. The largest projected application for itaconic acid is methyl methacrylate, which may account for 52.2% of the global itaconic acid market by 2020 [13]. Unsaturated polyester resins and detergent builders may account for 18.8% and 10.8% of global itaconic acid market in 2020. The main limiting factor to this is the relatively higher price of IA compared with potential alternatives such as acetone cyanohydrin. The same scenario applies with the potential for the replacement of maleic anhydride with IA in the production of unsaturated polyester resins (UPR) with fumaric acid as a competing monomer. Voll and Marquardt [19] also produced 3-methyltetrahydrofuran from IA but concluded that the process required a cheap source of IA to be feasible.

However Weastra et al. in the same report [13] posit that the production capacities of itaconic acid are not expected to grow dramatically, but in the event of increased demand, the production of

citric acid can be easily switched to itaconic acid. The report however adds the caveat that this might change if Lucite International, which already owns patents on the production of methyl methacrylate from IA, starts commercial production in 2016 as they have announced in partnership with Mitsubishi Rayon. Their goal to produce 50% of methyl methacrylate (approximately 400,000 tonnes) can result in significant increase in itaconic acid demand. Itaconix Corporation an American company which is a world leader in IA polymer production manufactures proprietary polymers used in chelation and builders as replacements for petro-based chemicals in detergents and water treatments.

Conclusion

Currently itaconic acid is predominantly produced by fermentations using several strains of *Aspergillus terreus*. Itaconic acid is a versatile chemical, which is used in the production of polymers with many desirable characteristics. Even though it can potentially replace petroleum-derived chemicals as a monomer in various polymerisation processes, the IA application is hampered by its relative high production cost. The most promising way to lower the cost of IA production is to reduce the cost of the substrate/raw materials by utilizing novel, cheap, non-food substrates. In addition, solid-state fermentation could also be exploited in bringing down the production cost.

Increasing the conversion yield of substrate to IA by improving the efficiency of the IA biosynthesis pathways will also lower the cost. Genetic manipulation of *E. coli* and the prolific citric acid producer *Aspergillus niger* may prove to be invaluable in this regard. Furthermore, the exploration of more efficient product recovery methods will help to minimize product losses and thus bring down the cost of production.

In conclusion, if the widespread plans to minimize the environmental impact caused by the use of petro-based products by replacing them with bio-based drop-ins or replacements become a reality, then itaconic acid production by microbial fermentations will witness rapid advancements.

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