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BIOSYNTHESIS OF ITACONIC ACID. A REVIEW

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ABSTRACT

Itaconic acid (IA) is an acrylic acid used for the production of petrochemical based products these days. CadA is an important molecule in the production of IA. *Aspergillus terreus* is highly known for its production as it yields 91 g/L itaconic acid by utilizing carbohydrate substrate. With the depletion of fossil fuels, scientists have started to produce such a compound obtained from microorganisms which will fulfill the need. *Pseudozyma Antarctica, Ustilago maydis* and *Candida* have also been reported for its production but the amount of IA produced is not that much enough. The demand of IA is readily increasing because it's a biodegradable product and its need will be increased more till 2020. China is the leading producer of IA. This review focus on latest progress in production of IA by using improved strains, its production from cheaper sources, applications and its future perspective.

KEYWORDS: Itaconic acid, *Aspergillus terreus*, glucose, China, global production

INTRODUCTION

With the advancement of technology, chemical industry has decided to replace petroleum based compounds with the naturally produce compounds. The well-known example is of itaconic acid which is emerging these days. Fig.1 shows the structure of itaconic acid.

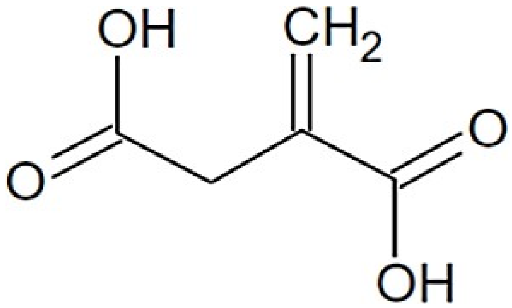

Figure 1: Structure of itaconic acid.

Itaconic acid is actually a methacrylic acid or acrylic acid. It is found in white crystalline powder with unsaturated di-carbonic acid compound usually conjugated with methylene. This compound is stable in acidic, basic and neutral conditions at moderate temperature (Tate, 1981). It is also isomeric with mesaconic and citraconic acid. It shows best adhesive and stability by latex because of the presence of two carboxylic groups. Moreover, it is also been used in the production of artificial glass (Kin *et al.,* 1998) and also in many compounds use in pharmacy, agriculture and medicine. It is also used in the formation of poly-functional building blocks by enzymatic transformations. Still these days; coal, crude oil and natural gas are in use for the massive production of chemicals. As these primary raw materials are used, there has been concern about the eco-friendly environment and also the energy shortage has caused to search for other viable sources to replace these primary raw materials. Because of global warming, plant biomass is thought to be best in use as it produces less carbon in the environment. It is also found that many compounds including propane-diol (Du *et al.*, 2007), succinic acid (Lin *et al.*, 2011) and ethanol (Pensupa *et al.*, 2013) are no being produced from renewable biomass. Itaconic acid has the enormous range of products and applications which will help to replace petroleum and also the negative effects of petroleum based compounds on the environment. Itaconic acid is produced by the fermentation of *A. terreus* mostly and its production with east and bacteria is not that much reported. For the production of enzymes and secondary metabolites, different microbes are being used. For the production of desired product, culture medium is being optimized and usually carbon source; sucrose or glucose is used. It is found that mycelia growth of *A. terreus* is sensitive to phosphate limitation and also to the availability of nitrogen and carbon. It was also noted that other species including *Rhodotorula* sp., *U.maydis*, and *Candida* sp. produced itaconic acid. But it is still noticed that *A. terreus* is the chief producer of itaconic acid which yields 80-86 g/L (Okabe *et al.*, 2009). It still demands for the lower price of the starting material of itaconic acid in future. In order to meet the future requirements of fuels, it is necessary that these requirements must not compete with the food chain. So it is highly recommended to use nonedible resources for these prespective. It is found that itaconic acid which is a five carbon unsaturated dicarboxylic acid is used these days for the production of itaconic acid (Werphy & Peterson, 2004).

In the past, itaconic acid was produced by different methods including oxidation of mesityl oxide which led to the isomerization of citric acid, destructive distillation of citric acid, oxidation of isoprene, propargyl method and heating the calcium aconitate. It was first reported by Kinoshita in1932 that the itaconic acid was isolated from the fungi *A. itaconicus*. It was thought that itaconic acid was first produced as a metabolite in the strain of *Aspergillus* which named as *A. itaconicus* (Kinsoshita *et al.*, 1932). But now a days, immobilized cells and novel batch strategies have been under consideration for the production of this acid.

Properties of itaconic acid

The stoichiometric formula of itaconic acid is $C_5H_6O_4$ and its molecular weight is 130.1 g/mol. It is also known as methylenebutanedioic acid (Pomogailo *et al.*, 2010). It was firstly discovered as a product of citric acid by Baup in 1836. It is in the form of white to light beige crystals. It has a density of 1.573 g/ml at 25°C. Itaconic acid has a M.p of 165-168°C T. The purification of itaconic acid as crystals is quite easy because it dissolves up to 80.1 g/L. The solubility of itaconic acid increases with the increase in temperature and it also dissolves in organic solvents like ethanol and methanol (Yang *et al.*, 2012). Because of its biodegradable property, it is an important polymer which is responsible for polymerization. It is oxidized than glucose with a noticeable value of 4 (Tomic *et al.*, 2010). It is twice acidic and reactive than maleic and fumaric acid (Fink, 2013). It also reacts with the amines as well and used in shampoos, pharmaceuticals, detergents and herbicides. Recently, two new derivatives of itaconic acid have been found as a metabolite from *A. aculeatus* namely 9-hydroxyhexylitaconic acid-4-methyl ester and 9- hydroxyhexylitaconic acid (Antia *et al.*, 2011).

Pathway of itaconic acid

It was debate that how itaconic acid had been arisen, either from TCA cycle or from condensation of acetyl-CoA. Two notable scientists proposed a pathway which shows that it starts from a carbohydrate substrate and has been proceed through glycolysis to pyruvic acid as shown in Fig. 2. As it splits down, carbon is metabolized to a product known as Acetyl-CoA which results in releasing CO_2. In the citric acid pathway, molecule known as CadA forms itaconic acid in the last step and releases CO_2. By using C_{14} and C_{13} substrates labeled, this pathway was traced (Winskill, 1983).This pathway deals with the intermediate metabolites and also use enzymatic abilities as well. The process of compartmentalization was also checked by fractions of cell extracts. The actual key enzyme is located in the cytosol (Jaklitschetal, 1991). In the recent research (Voll *et al.*, 2012), it is found that CadA is important toward the itaconic acid pathway. Itaconic acid is also noted in mammalian cells present in macrophage derived cell (Strelkoetal, 2011). This is because these cells shown the

CadA activity and produce itaconic acid.

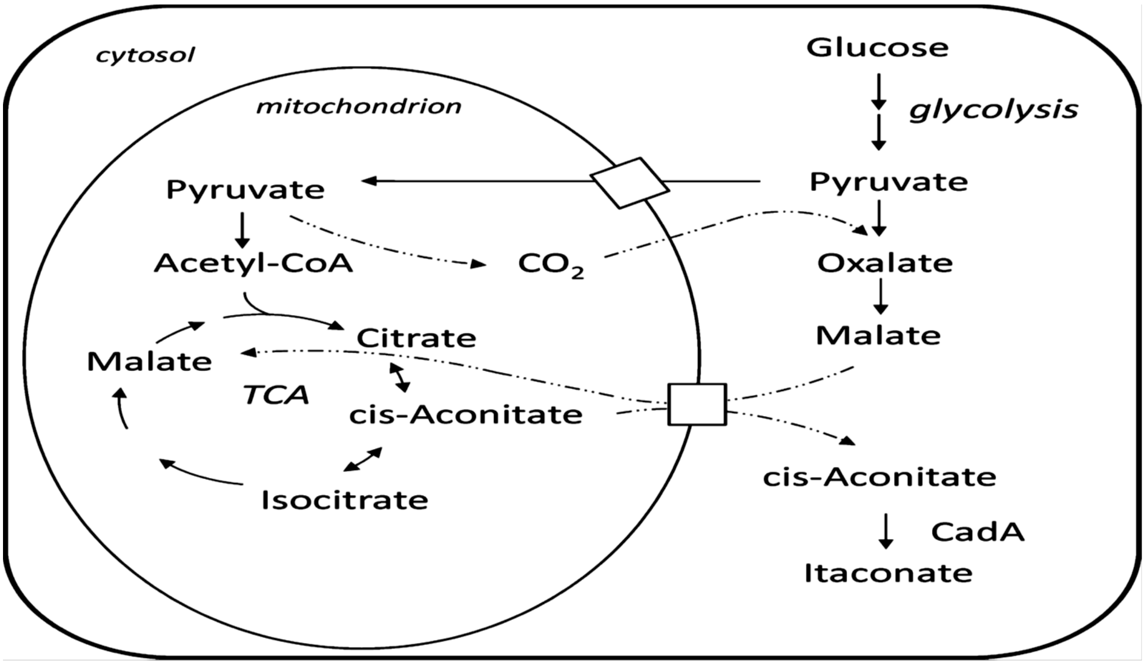

Figure 2: Biosynthesis of itaconic acid.

Microorganisms

For production of itaconic acid a large number of microorganisms were screened and studied. Through many trials of researches it was concluded that *A. terreus* gives more yield of itaconic acid than *A. itaconicus*. The most commonly used strain of *Aspergillus* for itaconic acid production is *A. terreus* NRRL 1960. This strain is stored under different names in culture stores like WB 1960, IAM 2054, QM 6856, CBS 116.46, IFO 6123 and IMI 44243. This strain is most important because it has the ability to produce almost 91 g/L itaconic acid using glucose as carbon source (Kuenz e*t al*., 2012). Another strain *A. terreus* IMI 282743 gave 5.76 g/L yield of itaconic acid using effluent of palm oil mill (Jahim *et al*., 2006). These were are native strains giving higher yield of itaconic acid but in industry more yield was required so strain improvement procedures have been adapted by researchers and native strains are genetically modified.

Strain improvement

Two processes have been used for improvement of itaconic acid production which are strain mutagenesis and genetic modification. Yahiro and his coworkers took wild *A. terreus* strain IFO6365, mutated it and got new modified *A. terreus* strain TN484-M1 which had ability to produce almost 82 g/L itaconic acid in 6 days fermentation (Yahiro *et al*., 1995). The reason of enhanced performance is considered to be stronger transcription in mutant strain of a gene

Cad1 which encode cis-aconitic acid decarboxylase (Kanamasa *et al*., 2008). Then experimentation continued and Dwiarti and his colleagues used that IFO6365 strain and changed substrate and used sago starch to produce itaconic acid and got 48.2 g/L yield (Dwiarti *et al*., 2007). Then Reddy and Singh did mutagenesis of *A. terreus* native strain SKR10 with help of chemical mutagens and ultraviolet rays in several experiments. As a result they got two strains which improved than native strain. One was *A. terreus* N45 and other was designated as *A. terreus* UNCS1. Former gave yield 50 g/L using corn starch as carbon source while latter gave 32 g/L itaconic acid from extracts of fruit wastes. These amounts were comparatively higher than native strain from same substrates (Reddy & Singh, 2002).

The effect of over expression of cadA, mfsA, mttA and ATEG-09969 in *A. terreus* on itaconic acid production is described in the above Fig. 3. Cad-A-21 produced IA upto 88.1 g/L and it showed that this production is beneficial for IA production. Cad gene actually contributed to IA production.

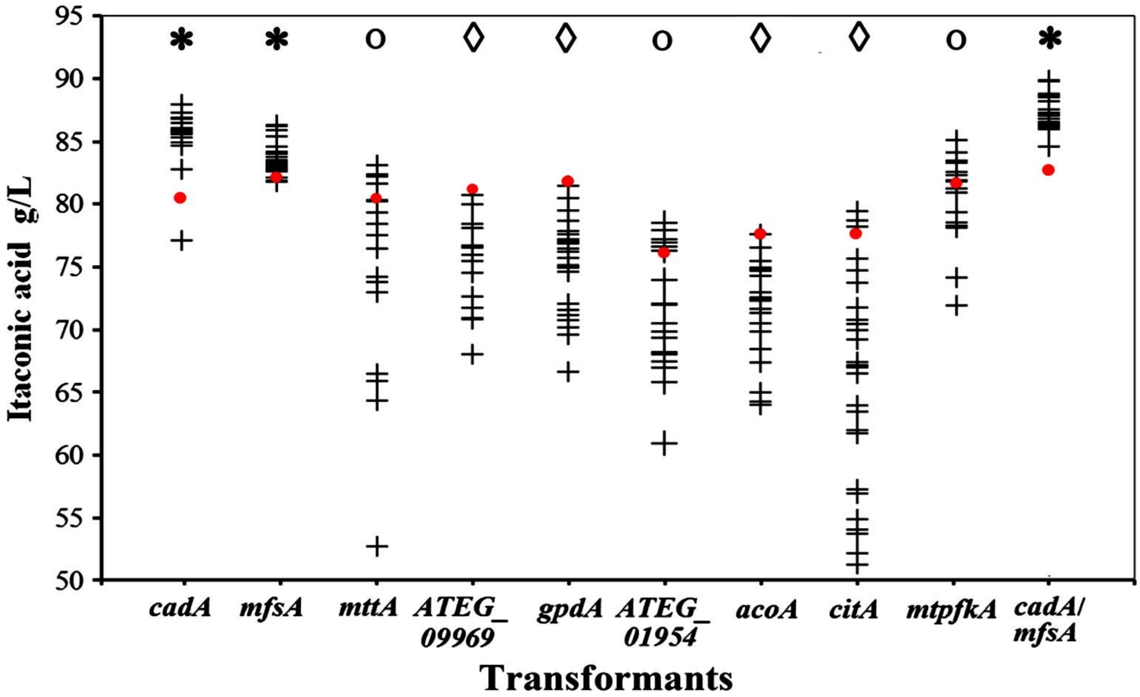

Figure 3: Itaconic acid production by transformants of genes.

Many other microorganisms were also reported to produce itaconic acid but those all couldn't be considered ideal unless they have ability to exceed or even equalize the yield of itaconic acid by *A. terreus* or they are easy to modified genetically to get desirable characteristics, are not too complex to handle and have least by product formation. All these desirable characteristics can be introduced into host microorganism. Many fungal and yeast strains have been reported to give itaconic acid production other than *A. terreus* and *A. itaconicus*. Itaconic acid was produced as by-product by an *Ustilago zeae* strain during its fermentation process for production of ustilagic acid (Haskins *et al*., 1955). Then *Ustilago maydis* was reported to produce itaconic acid using glucose as carbon source. Iwata corporation Japan reported 53 g/L yield of itaconic acid by *Ustilago maydis* (Voll *et al*., 2012). Then Levinson and his colleagues reported production of itaconic acid from fermentation of a basidiomycete fungi *Pseudozyma antarctica* and they reported the yield almost 30 g/L (Levinson *et al*., 2006). A yeast strain *Candida* was also reported to produce itaconic acid after many trials of experiments and mutations. That strain gave 53 g/L yield of itaconic acid in 5 days (Tabuchi, 1981).

A new strategy was proposed by Tkacz and Lange to introduce cisaconitate decarboxylase gene in *A. niger* strains which were used for citric acid production to get itaconic acid (Tkacz & Lange, 2004). That strategy was then used by Li and coworkers to produce genetically transformed *A.niger* and reported 0.13 g/L itaconic acid production. Just like genetically transformed itaconic acid producing fungi strains, bacterial strains were also reported to be modified for itaconic acid production for example *E.coli* and *Saccharomyces cerevisiae* (Blazeck *et al.*, 2014), *Corynebacterium glutamicum* (Otten *et al.*, 2015), and *Yarrowia lipolytica* (Blazeck, 2015).

Substrates for the production of itaconic acid

Most common substrate used by *A. terreus* for itaconic acid production is glucose (Reddy & Singh, 2002). Sucrose is also used as a good substrate. Arabinose was tested but yield was low as 18% while xylose gave yield 31% which were quite low as compared to glucose and sucrose. Lactose gave even less 1% yield so these are not preferred. Other sugar sources from food sources like wheat, sago, cassava, sorghum and sweet potato were also used for itaconic acid production as shown in Table 1. Due to increase in price of glucose and sucrose, alternative cheaper substrates are more beneficial to use in production of itaconic acid. These may be agro- wastes and organic acids. Agro-wastes are first treated through various methods and then made capable for use by *A. terreus*. Examples of agro-wastes include corn syrup, jatropha seed cake and molasses (Klement *et al.*, 2012; Alvira *et al*., 2010).

Table 1: Itaconic acid production from substrates adapted from Kuenz & Kruell, 2018.

Substrate	**Purification**	**Microbes**	**IA acid g/L**	**Yield g/g**	**Productivity g/L/h**	**Type**	**References**
Fruit waste (banana)	-	*A.terreus* Mutant N- 45	30	0.35	0.21	SF	Reddy & Singh, (2002).
Fruit waste (apple)	-	*A.terreus* ant N- 45	12	0.37	0.22	SF	Reddy & Singh, (2002).
Wheat bran hydrolysate	Neutralization $Ca(OH)_2$	*A.terreus* CICC40205	8	-	0.29	STR	Wu *et al.* (2017).
Wheat bran hydrolysate	Neutralization $Ca(OH)_2$	*A.terreus* CICC40205	34.2	0.41	0.41	STR	Wu *et al.* (2017).
Wheat bran hydrolysate	Neutralization $Ca(OH)_2$	*A.terreus* CICC40205	49.6	0.55	0.16	STR	Wu *et al.* (2017).
Corn stover hydrolystae	-	*A.terreus* CICC2452	0.64	0.2	0.009	SF	Li *et al.* (2016).

SF= Shake flask; STR=Stirred Tank reactor

But there are several issues linked to these agro-wastes like yield is lower than sugars, substrates have complex structures, they vary in their composition, presence of harmful chemicals in them and increased amount of impurities which make these processes relatively longer and complex than crude sugars. Glycerol and lignocellulose are also used as alternative substrates of sugars in fermentation price. Sources other than carbon like nitrogen, hydrogen and macronutrients, micronutrients are also important in fermentation of itaconic acid. Nitrogen source is very important for better yield of itaconic acid along with other trace metals. Zinc and iron play vital role in improvement of yield of itaconic acid (Lockwood & Reeves, 1945). Amount of Phosphate ion is kept low after development of mycelia to avoid carbon diversion into further production of mycelia. Amount of minerals and ions are kept optimum for better production of itaconic acid.

Conditions required for IA production

Temperature and fermentation time

Optimum temperature for itaconic acid production is usually 37°C (Willke & Vorlop, 2001). Attempts had been made to change this temperature to get better results and some researches proved that production was increased 5 times in mutant organisms when temperature was raised to 40°C. Fermentation time is also important in production of itaconic acid. Many researches have been done varying the time range from 2 to 14 days. But best yield was obtained in mean time of 7 days thus it is considered as optimum time duration of production of itaconic acid.

Dissolved oxygen

Fermentation of itaconic acid always involves aerobic microorganisms and thus oxygen supply really matters a lot in fermentation process. Similarly agitation is also important in fermentation process. There are different agitation speeds referred by different scientists. Park and his coworkers suggested that yield was improved when agitation speed was 320 rpm or impeller speed 0.94 m/s where yield per unit of consumed glucose was higher keeping dissolved oxygen 20% of saturation point (Park *et al.,* 1993). Similarly in other experiments Rychtera found that when we keep impeller speed at 0.71m/s then optimum aeration rate was achieved to get high itaconic acid yield (Rychtera & Wase, 1981). While Riscaldati in his experiments reported that by increasing agitation upto 400rpm or impeller speed 1.57m/s, itaconic acid production was raised (Riscaldati *et al.,* 2000).

Oxygen supply really matters a lot in fermentation process. Even a short cutoff of oxygen supply can damage mycelia and fermentation can be suppressed at high rate (Gyamerah, 1995). Thus a little interruption in oxygen supply can lead to great loss. Then Vitreoscilla haemoglobin gene was reported which if present in culture has ability to overcome short interruptions of oxygen and this process somehow succeeded like transformants showed decrease in 39.2% in itaconic acid production while wild strain was showing 51.2% decrease in itaconic acid production in short supply of oxygen. This showed that transformants had 8 times more uptake of oxygen than native strain (Lin *et al.,* 2004).

Aeration rates in fermentation of *A. terreus* for itaconic acid production vary and different optimum values are suggested by different researchers using different strains. Thus optimum value depend upon strain which is being used and fermentation system which is being adapted because high impeller speeds can cause sheer stress on mycelia while low impeller speeds can cause poor supply of oxygen to mycelia. But if we increase overall aeration rate, it can lead to enhanced production but production cost will also increase thus every fermentation process needs to set its optimum dissolved oxygen at specific impeller speed.

Immobilization

Immobilized microorganisms are also used in fermentation for itaconic acid production. Polyacrylamide gel is the first ever matrix which was used for immobilization in itaconic acid production (Horitsu *et al.,* 1983). Then other matrices were also tested and used for this purpose. Those include polyurethane tubes, porous disk reactor system, structural fibrous network of pawpaw trunk wood and silica based materials. Then it was found that pore size of polyurethane foam didn't impart any effect on rate of loading of *A. terreus* and through this process Vassilev and his colleages obtained 15.1 g/L yield of itaconic acid with a carrier over 5 cycles.

Solid state fermentation

The agrowaste may be used primarily as mechanical support or as an anchor for an organism that situation, or it might comprises of most of the nutrients mandatory for the fermentation. 44 g/L IA from the desiccated olive wastes was produced by Vassilev though in a patented SSF method exploiting a mutant a yield of 55 g/L IA was attained. Moisture is a very important factor in SSF with an optimum level of 65% - 70% reported for IA production from agricultural wastes. Moisture values beneath this optimum, outturn in low nutrient solubility and a decrease in beet pr essmud swelling that was used as a substrate while at levels above 7% the air was distorted, so harmfully affecting the permeability and particle accumulation of the substrate. Because the optimal temperatures for IA fermentation are substantially higher than atmospheric, the substrate bed must be heated to the optimum temperature.

Aeration has the dual function of providing cells with oxygen to avoid hotspots by recirculating heat. In many bio-chemical processing it is widely believed that SSF can deliver better or equivalent yields in the context of submerged fermentation. Theory shows that the IA production process is ideally beneficial to SSF conditions, as strictly aerobic process that occurs at high temperatures and utilizes a filamentous fungus. Recently, SSF has increased interest, especially in the use of agricultural waste as substrates. However, further optimization of fermentation conditions is still necessary.

Submerged fermentation

The conditions for the production of IA by *A. 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.

Production steps of IA

Steps involved in basic industrial production of itaconic acid which includes filtration of culture, removal of mycelia, concentration of product, decolorization, crystallization and packing. For high quality of purification, ion exchange method can be used. Recovery of itaconic acid in each step is such that 95% in filteration process, 98% in 2nd step of concentration, 95% in 3rd step of crystallization and drying. Total yield is estimated to be 80% from start of cultivation to end step that is packaging (Okabe *et al.*, 2009).

Down streaming process

Itaconic acid can be recovered relatively easily as it easily crystallizes. The fermentation liquor is distilled to extract suspended solids and mycelia after depletion of fermented sugars. The liquor is vaporized and then crystallized to about one fifth of its actual volume. These crystals are eroded out to remove impurities and recrystallized. Itaconic acid can also be precipitated by means of salts of metals like calcium and lead. As equated to the salt of calcium, the precipitation of IA, which is almost insoluble in air, as opposed to calcium chloride, is not pre- concentrated in broth. Lead is better to calcium salt precipitation as the lead salt of IA is usually not soluble in liquid being more resolvable than for instance calcium citrate. While itaconic acid has been recovered from model solutions using electrodialysis, there is still a very small amount of information available about its membrane separation as a means of IA recovery.

Applications

Some of the products namely, styrene-butadiene rubber, polyitaconic acid and butadiene can be made from itaconic acid as shown in Table. 2. These days unsaturated polyester resins are also produced by IA. For non-woven fiber binders, itaconic acid is used with the combination of acrylate latexes. Similarly, acrylonitrile and itaconic acid copolymer is also makes dying easier (Vol & Marquaedt, 2012). In order to enhance the adhesion ability of paints, itaconic acid is used. 5% of itaconic acid is mixed in acrylic resins to hold ink in the process of printing. Itaconic acid also shows analgesic properties because of its esters and also exhibits growth activities of plant (Leucia *et al.,* 2006). For the synthesis of some biofuels namely 2-methylbutanediol and 3- methyltetrahydrofuran, itaconic acid is used (Klement *et al.,* 2012). It also shows a high affinity as monomer to deoxynivalenol (DON) which is actually a mycotoxin of specie *Fusarium* (Pascale *et al.,* 2008).

Market demand of IA

It is thought that Global market for itaconic acid will be exceeded to US $133 in 2024 because of the shift from petrochemicals to bio based resources. Because of the growing demand of products, fossil fuels have been depleting and there is a fast research going on the production of such product which overcomes this need and itaconic acid is one of them. Itaconic acid is used in different ways which includes consumption of itaconic acid in super absorbent polymers, use of synthetically produced rubber in mats and flooring, used as a substitute of sodium tripolyphosphate, polyester resins, in the production of biocompatible implants and in feedstock. Its market demand is keep on increasing because it is biodegradable and results in reduced amount of industrial waste. In 2011, global production was 41,000 ton of price $74.5 million and thought to exceed up to 50,000 ton of price $547 million up to 2020. China is the largest producer of itaconic acid production with an annual capacity of 20,000 ton. China is also in the list of four countries which produces IA. The demand of IA is exceeded in 2005. The price of IA is US $ 2/kg as compared to the last ten years when the price was US $4/kg (Willke & Vorlop., 2001). The price has been fallen in China because of the restricted range of applications.

Table 2: Applications of IA.

Materials	**Applications**	**References**
Rubber like resin	Electrical insulation	Smith *et al.* (1974).
Hardening agent	Contact lens	Ellis *et al.* (1994).
Styrene bytadiene	Carpet backing	Willke & Vorlop, (2001).
Acrylic lattices	Non-woven fiber binder	Willke &Vorlop, (2001).
Sulfonated poly IA	Industrial cleaner	Willke & Vorlop, (2001)
Polyhydrogels	Drug delivery	Stanojevic *et al.* (2006).
Polyhydrogels	Glass ionomer cements	Culbertson, (2006),

Table 3: Suppliers of IA.

Company	**Location**	**Since**	**Tons/year**
Rhodia	Melle, France	1995	10,000
Iwata Chemicals	Kyogyo,Japan	1970	10,000
Pfizer food Science	New York	1945-1999	5000-7000
Qingdao Langyatia Yueli IA Co.Ltd	Guangdong, China	1999	1500
Tianli Biological Fermentation factor	Yunnan, China	1988	2000
Gansu Fiepeng Biochemical Co.Ltd	Gansu, China	1989	1000
Qingdao Langyatai Group	Qingdao, China	2000	4500

Trends in IA production

After analyzing the publications, it can be referred that the interest in it is decreasing as shown in Fig. 4. Following the report by DoE which shows IA as a promising chemical there is a renewed interest in its publication.

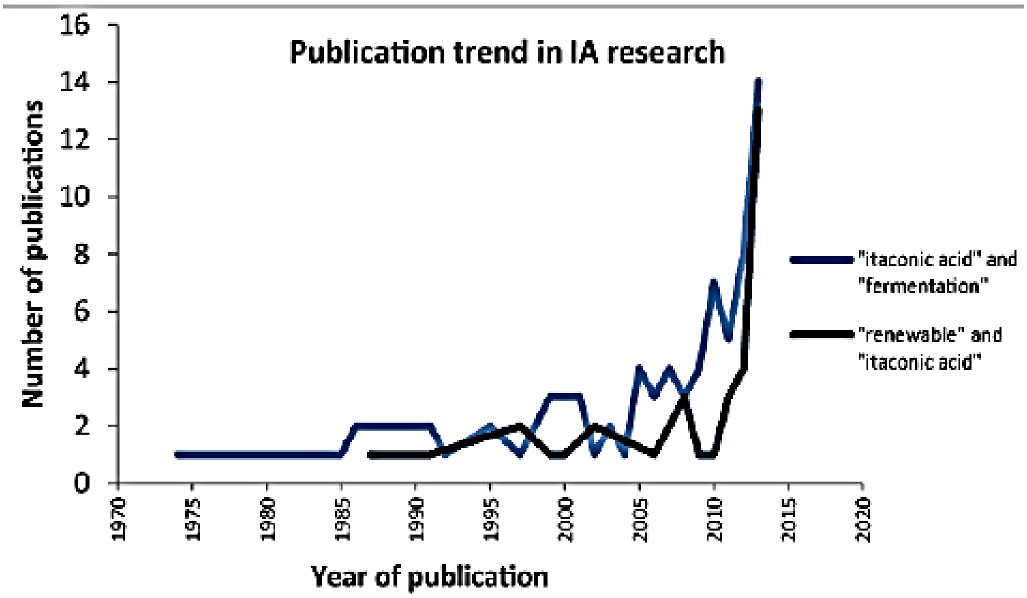

Figure 4: Publication trend in IA production.

CONCLUSION

IA is a sensational chemical used in the production of variety of polymers with the required characteristics. It is also utilized to replace petroleum derived chemicals in the polymerization process as a monomer. Glucose is used for the high production rate of IA by *Aspergillus terreus*. But in the present era, lignocellulosic material can be used as a cheaper source for its production. Although, strain improvement and genetically modified organism techniques also made the best production of IA. Currently, its cost has been decreased by the use of cheap raw materials and also by the use of solid state fermentation strategy. Another approach to decrease the cost of IA production is to improve the biosynthesis pathway efficiently. It is also seen that genetic manipulation of *E. coli* is invaluable in this process. With the advancement in technology, the usage of more efficient methods help in decreasing the product loss and also rings down the productivity cost of itaconic acid.

Conflict of interest

There is no conflict of interest regarding to this article.

REFERENCES

1. Alvira, P., E. Tomas Pejo, M.Ballesteros and M.J. Negro. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Biores Technol*, 2010; 101: 4851-4861.
2. Antia, B. S., T. Aree, C. Kasettrathat, S. Wiyakrutta, and O. D. Ekpa. Itaconic acid derivatives and diketopiperazine from the marine-derived fungus *Aspergillus aculeatus*

CRI322-03. *Phytochem.*, 2011; 72: 816-820.

3. Blazeck, J., A. Hill, M. Jamoussi, A. Pan, J. Miller and H. S. Alper. Metabolic engineering of *Yarrowia lipolytica* for itaconic acid production. *Metab Eng.*, 2015; 32: 66–73.
4. Blazeck, J., J. Miller, A. Pan, J. Gengler, C. Holden, M. Jamoussi and H. S. Alper. Metabolic engineering of *Saccharomyces cerevisiae* for itaconic acid production. *Appl Microbiol Biotechnol.*, 2014; 98: 8155–8164.
5. Culbertson, B.M. New polymeric materials for use in glassionomercements. *J Dent.*, 2006; 34: 556–565.
6. Du, C., Y. Zhang, Y. Li and Z. Cao. Novel redox potential-based screening strategy for rapid isolation of *Klebsiella pneumoniae* mutants with enhanced 1,3-propanediol-producing capability. *Appl and Environ Microbiol*, 2007; 73: 4515-21.
7. Dwiarti, L., M. Otsuka, S. Miura, M. Yaguchi and M. Okabe. Itaconic acid production using sago starch hydrolysate by *Aspergillus terreus* TN484-M1. *Bioresour Technol,* 2007; 98: 3329-3337.
8. Ellis, E.J, A. P. Olson and J. R. Bonafini. Improved itaconic acid copolymeric compositions for contact lenses. *WO Patent.*, 1994; 9: 314-423.
9. Fink, J.K. Reactive polymers fundamentals and applications: a concise guide to industrial polymers. William Andrew Publishing, Norwich, 2013.
10. Gyamerah, M. Factors affecting the growth form of *Aspergillus terreus* NRRL 1960 in relation to itaconic acid fermentation. *Appl Microbiol and Biotechnol.*, 1995; 44: 356–361.
11. Haskins, R.H., J. A. Thorn and B. Bothyrod. Biochemistry of the Ustilaginales. XI. Metabolic products of *Ustilago zeae* in submerged culture. *Can J Microbiol.*, 1955; 1: 749-756.
12. Horitsu, H., Y. Takahashi, J. Tsuda and Y. Kawano. Production of itaconic acid by *Aspergillus terreus* immobilized in polyacrylamide gels. *Appl Microbiol and Biotechnol,* 1983; 18: 358-360.
13. Jahim, J. M., N. I. S. Muhammad and W. T. Yeong. Factor analysis in itaconic acid fermentation using filtered POME by *Aspergillus terreus* IMI 282743. *Jurnal Kejuruteraan*, 2006; 18: 39-48.
14. Jaklitsch, M., P. Kubicek, P. Scrutton, C. London, C. H. Road, and W. London, W. Thesubcellular organization ofitaconatebiosynthe- sis *Aspergillusterreus*. *J. Gen. Micro-boil*, 1991; 137: 533–539.

15. Kanamasa, S., L. Dwiarti, M. Okabe, E. Y. Park. Cloning and functional characterization of the cis-aconitic acid decarboxylase (CAD) gene from *Aspergillus terreus. Appl Microbiol Biotechnol,* 2008; 80: 223-229.
16. Kin, R., T. Sai and S.So. Itaconate copolymer with quadratic nonlinear optical characteristic. *JP Patent No.,* 1998; 10: 293,331.
17. Kinoshita, K. Uber die Produktion von Itaconsaure und Mannit durch einen neuen Schimmelpilz *Aspergillus itaconicus*. *Acta Phytochim*, 1932; 5: 271–287.
18. Klement, T., S. Milker S, G. Jager, P. M. Grande and P. Dominguez de Maria. Biomass pretreatment affects *Ustilago maydis* in producing itaconic acid. *Microb Cell Fact,* 2012; 11: 43.
19. Kuenz, A and S. Krull. Biotechnological production of itaconic acid—things you have to know. *Appl microbioland biotechnol,* 2018; 102(9): 3901-3914.
20. Kuenz, A., Y.Gallenmüller, T.Willke and K.D. Vorlop. Microbial production of itaconic acid: developing a stable platform for high product concentrations. *Appl Microbiol Biotechnol.*, 2012; 96: 1209-1216.
21. Lai, L.S., C. S. Hung and C.C. Lo. Effects of lactose and glucose on production of itaconic acid and lovastatin by *Aspergillus terreus* ATCC 20542. *J Biosci Bioeng*., 2007; 104: 9-13.
22. Levinson, W.E, C.P. Kurtzman and T.M. Kuo. Production of itaconic acid by *Pseudozyma antarctica* NRRL Y-7808 under nitrogen-limited growth conditions. *Enzy and Micro Technol.*, 2006; 39: 824-827.
23. Li, A., N. Pfelzer, R. Zuijderwijk and P. Punt. Enhanced itaconic acid production in *Aspergillus niger* using genetic modification and medium optimization. *BMC Biotechnol*, 2012; 12: 57.
24. Li, X., K. Zheng, C. Lai, J. Ouyang and Q. Yong. Improved itaconic acid production from undetoxified enzymatic hydrolysate of steamexploded corn stover using *an Aspergillus terreus* mutant generated by atmospheric and room temperature plasma. *Bioresources*, 2016; 11(4): 9047–9058.
25. Lin, C.S.K., R. Luque, J. H. Clark J and C. Du. A seawater-based biorefining strategy for fermentative production and chemical transformations of succinic acid Energy & Environmental Science, 2011; 4: 1471-1479.
26. Lin, Y.H., Y. F. Li, M. C. Huang and Y. C. Tsai. Intracellular expression of Vitreoscilla hemoglobin in *Aspergillus terreus* to alleviate the effect of a short break in aeration during culture. *Biotechnology letters*, 2004; 26: 1067-1072.

27. Lockwood, L.B and M.D. Reeves. Some factors affecting the production of itaconic acid by *Aspergillus terreus. Arch of Biochem,* 1945; 6: 455– 469.
28. Lucia, L. A., D. S. Argyropoulos, L. Adamopoulos and A. R. Gaspar. Chemicals and energy from biomass. *Canad. J. of Chem,* 2006; 84: 960-970.
29. Milsom, P. E. and J. L. Meers. Gluconic and itaconic acids. Comprehensive biotechnology: the principles, applications, and regulations of biotechnology in industry, agriculture, and medicine. *Angewandte Chemie International,* 1985; 26: 588.
30. Okabe, M., D. Lies, S. Kanamasa and E. Y. Park. Biotechnological production of itaconic acid and its biosynthesis in *Aspergillus terreus*. *Appl microbiol and biotechnol*, 2009; *84*(4): 597- 606.
31. Otten, A., M. Brocker and M. Bott. Metabolic engineering of *Corynebacterium glutamicum* for the production of itaconate. *Metab Eng,* 2015; 30: 156–165.
32. Park, Y., N. Ohta N and M. Okabe. Effect of dissolved oxygen concentration and impeller tip speed on itaconic acid production by *Aspergillus terreus*. *Biotechnology letters,* 1993; 15: 583-586.
33. Pascale, M., A. D. Girolamo, A. Visconti, N. Magan and I. Chianella. Use of itaconic acid-based polymers for solid-phase extraction of deoxynivalenol and application to pasta analysis. *Anal Chim Acta,* 2008; 609: 131-138.
34. Pensupa, N., M. Jin, M. Kokolski, D. B. Archer and C. Du. A solid state fungal fermentation-based strategy for the hydrolysis of wheat straw. *Bioresour Technol,* 2013; 149: 261- 267.
35. Pomogailo, A. D., V. N. Kestelman and G. I. Dzhardimalieva. Monomeric and Polymeric Carboxylic Acids. In Macromolecular Metal Carboxylates and Their Nanocomposites. *Springer Berlin Heidelberg,* 2010; 7-25.
36. Reddy, C.S and R. P. Singh. Enhanced production of itaconic acid from corn starch and market refuse fruits by genetically manipulated *Aspergillus terreus* SKR10. *Bioresour Technol,* 2002; 85: 69-71.
37. Riscaldati E, Moresi M, Federici F, Petruccioli M Effect of pH and stirring rate on itaconate production *by Aspergillus terreus. J Biotechnol,* 2000; 83: 219-230.
38. Rychtera, M and D. A. Wase. The growth of *Aspergillus terreus* and the production of itaconic acid in batch and continuous cultures. The influence of pH. *J of Chem Technol and Biotechnol,* 1981; 31: 509-521.
39. Smith, J. E., A. Nowakowska-Waszczuk and J. G. Anderson. Organic acid production by mycelial fungi. *Indus aspects of bioche,* 1974; 297–317.

40. Smith, J. E., A. Nowakowska-Waszczuk and J. G. Anderson. Organic acid production by mycelial fungi.*Indus aspects of bioche,* 1974; 297–317.
41. Stanojevic, M., M. K. Krusic, J. Filipovic, J. Parojci and M. Stupar. An investigation into the influence of hydrogel composition on swelling behavior and drug release from poly (acrylamide-coitaconic acid) hydrogels in various media. *Informa Pharm Sci*, 2006; 13: 1–7.
42. Steiger, M. G., M. L. Blumhoff, D. Mattanovich and M. Sauer. Biochemistry of microbial itaconic acid production. *Frontiers in microbiology*, 2013; *4*: 23.
43. Tabuchi, T. Itaconic acid fermentation by ayeast belonging to the genus *Candida*. *Agricul and BiolChem.*, 1981; 45: 475-479.
44. Tate, B.E. Itaconic acid and derivatives. Grayson MEckroth E (eds) Kirk-Othmer Encycl.*Chem Technol*, 1981; 3: 865–873.
45. Tkacz, J.S and L. Lange. Advances in fungal biotechnology for industry, agriculture, and medicine. Springer, 2004.
46. Tomic, S. L., M. M. Micic, S. N. Dobic and E. H. Suljovrujic. Smart poly (2-hydroxyethyl methacrylate/itaconic acid) hydrogels for biomedical application. *Radiat Phy and Chem*, 2010; 79: 643-649.
47. Voll, A and W.Marquardt. Reaction network flux analysis: Optimization-based evaluation of reaction pathways for biorenewables processing. *AIChE Journal,* 2012; 58: 1788- 1801.
48. Voll, A., T. Klement, W. G. Gerhards. Metabolic modelling of itaconic acid fermentation with *Ustilago maydis*, Chem Eng, 2012; 27.
49. Voll, A., T. Klement, T. Gerhards, G. J. Büchs J., and W. Marquardt. Metabolic modeling of ita-conic acid fermentation with *Ustilago maydis*. *Chem. Eng. Trans*, 2012; 27: 367–372.
50. Werphy, T and G. Peterson. Top value added chemicals from biomass. Results of screening for potential candidates from sugars and synthetic gas. US Department of Energy, 2004; 1: 1–76.
51. Winskill, N. Tricarboxyli cacid cycle activity in relation of itaconic acid biosynthesis by *Aspergillus terreus*. *J. Gen.Microbiol,* 1983; 129: 2877– 2883.
52. Xuenian, H., L. Xuefeng, L. Yueming, L. Xia and J. J. Li. Improving itaconic acid production through genetic engineering of an industrial *Aspergillus terreus* strain *Microbiol Cell Factories*., 2014; 13: 119.